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SORPTION STUDIES OF IONIC PESTICIDES AND HYDROPHOBIC ORGANIC COMPOUNDS ON POLYMERIN FOR POTENTIAL WATER REMEDIATION

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CHAPTER 1

General Introduction

1.1 Xenobiotics and Pollution

The production and subsequent storage or transportation of hazardous materials are integral part of our economy. Consequently, there is an enormous variety of synthetic organic chemicals (*xenobiotics*) which enter our environment. The term xenobiotic (from the Greek *xenos* "foreign" and *bios* "life") includes all those compounds which are foreign to living organisms. These man-made chemicals are deliberately or accidentally released in the environment by different ways, such as leakages or spillages from pipes and tanks; deposition of airborne emissions; storage and disposal of raw materials or unwanted wastes and residues; use of contaminated fill materials; application of sewage or industrial sludge to land and spraying of pesticides. In most instances, these problems involve the contamination of soil and/or groundwater and may also involve the contamination of sediments either on-site or in nearby water bodies. The American National Research Council (NRC) estimated that since the late nineteenth century approximately eight millions of organic xenobiotics have been widely disseminated in the environment (NRC, 2003). The negligent spreading of xenobiotics lead, inevitably, to point and non-point source pollution.

In accordance with Léon et al. (2001) and Mohaupt et al. (2000), point-source pollution is defined as the discharge of a discrete identifiable source,

such as a waste pipe, a stream by a wastewater treatment plant system or sewer overflows. These loads results, e.g., from disposal of pesticides and filling and cleaning of spraying equipment on farmyards during dry weather periods, as well as from washing off pesticide residues from impervious areas (farmyards, streets, roofs, etc.) during storm events. On the other hand, transport processes, such as soil surface runoff, interflow, preferential flow, leaching, atmospheric depositions and spray drift lead to non-point source pollution. The ability for pollutants to reach surface water or groundwater is enhanced by the amount of warecipitation (rain) or irrigation. The release of pollutants into the environment can kill organisms outright, change the biogeochemical conditions and processes occurring within a system and result in systemic changes that degrade habitats and make disorders in ecological processes. In rural areas, irrigation run-off from farming activities may sometimes contain insecticides, fertilizers and herbicides that have been applied to crops. This runoff may affect aquatic and marine organisms living in the catchments and their associated estuaries and in-shore marine ecosystems. Chemicals can accumulate in the food chain, particularly in the higher order animals, and can damage non-target populations if applied carelessly.

1.2 Pesticides and the Environment

A *pesticide* (or farm chemical, or agro chemical) is a substance or mixture of substances used for preventing, controlling, or lessening the damage caused by a pest (EPA, 2007). Using the name ‘pesticide’ we describe a range of products used to control insects, diseases and weeds. They include insecticides, fungicides, herbicides, molluscicides, plant growth regulators as

well as bird and animal repellents and rodenticides. Pesticides classification is shown in Table 1.

Table 1. Pesticides classification based on their target organism

PESTICIDE	***TARGET***
Insecticide	Insects
Fungicide	Mold, rust, anthracis
Herbicide	Mono and dicotyledons weeds
Nematocide	Nematodes
Molluscicide	Snails
Acaricide	Mites

Pests are those organisms like weeds, insects, bacteria, fungi, viruses and animals which can i) reduce the quality and quantity of food produced by lowering production and destroying stored produce; ii) harm our animals (like fleas, worms and diseases) and iii) compete with humans for food and affect the health, welfare and way of life of people.

A pesticide may be a chemical substance (antimicrobial and disinfectant) or a biological agent (viruses or bacteria). They may be derived from inorganic sources (copper, sulphur), natural organic sources (plants and microorganisms) or organic compounds synthesized in a laboratory, which mimic the activity of the natural ones.

Pesticides are used in many situations such as livestock farming, cropping, horticulture, forestry, home gardening, homes, hospitals, kitchens, roadsides, recreational and industrial areas. In the last 60 years chemical control has been widely used (Day et al., 1997) but many of the early chemicals had disadvantages because they were often highly toxic and very persistent, e.g., DDT (Sladen et al., 1996; Russel, 1999), posing a threat to the environment, or they damaged the crops they were meant to protect.

Modern pesticides are sophisticated compounds which are very carefully researched to ensure high specificity against target organisms, safer to the environment and utilizable without undue hazards to the operators or consumers. Many of these have been developed to target specific biochemical reactions within the target organism, e.g., an enzyme necessary for photosynthesis within a plant or a hormone required for normal development in an insect.

Commercial pesticides are composed by the substance on which the characteristic biocide action largely depends, or active principle, and by the excipients (inactive substances used as a carrier for the active ingredient or to improve the physico-chemical properties of the formulation). They are sold as powder, solution, emulsion, microencapsulate, in granular state or as a gas. When pesticides are used on crops or when animals are fed crops treated with pesticides, residues may remain in or on the food when it is sold. Before registering a pest control product for use, the government must determine that consumption of the residues that are likely to remain in or on the food when the pesticide is used according to label directions will not pose an unacceptable health risk. This amount is then legally established as a maximum residue limit (MRL). In the European Union, MRL in foodstuffs is 0.01 mg/kg. This general limit is applicable "by default", i.e., in all cases where an MRL has not been specifically set for a product or product type.

The widespread use and disposal of pesticides by farmers, institutions and the general public may provide, if not correctly managed, to their diffusion in the environment, where they may have many different fates. Pesticides may be released to farmyard surfaces from spillages, leakages (Higginbotham et al., 1999) and from washing and cleaning tractors and sprayers (Ramwell et al., 2004; Ramwell et al., 2007), and they can reach the water through different ways, as shown in Figure 1.

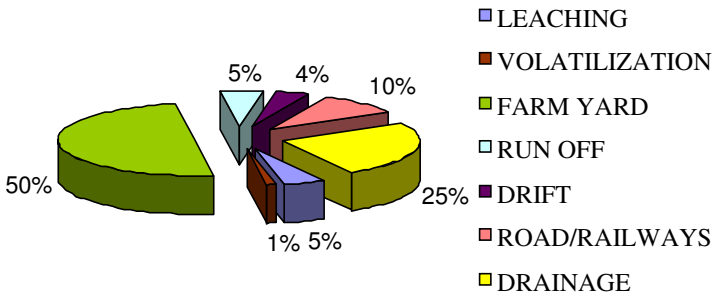


Figure 1. Sources of water contamination by pesticides.
(Modified from Mason et al., 1999).

Pesticides which are sprayed on the plants can move through the air and may eventually end up in other parts of the environment, such as in soil or water. Pesticides which are applied directly to the soil may be washed off the soil into nearby bodies of surface water or may percolate through the soil to lower soil layers and groundwater. The application of pesticides directly to bodies of water for weed control, or indirectly as a result of leaching from boat paint, runoff from soil or other routes, may lead not only to build up of pesticides in water, but also may contribute to air levels through evaporation.

This incomplete list of possibilities suggests that the movement of pesticides in the environment is very complex with transfers occurring continually among different environmental compartments. In some cases, these exchanges occur not only between areas that are close together (such as a local pond receiving some of the herbicide application on adjacent land) but also may involve transportation of pesticides over long distances.

Two things may happen to pesticides once they are released into the environment. They may be degraded by the action of sunlight, water or other chemicals, and/or microorganisms. This degradation process usually leads to the formation of less harmful breakdown products but in some instances can produce more toxic products. The second possibility is that the pesticide will be very resistant to degradation by any means and thus remain unchanged in the environment for long periods of time. The ones that are most rapidly broken down have the shortest time to move and have adverse effects on people and other organisms. The ones which last the longest, the so-called *persistent pesticides*, can move over long distances and can build up in the environment leading to greater potential for adverse effects to occur. In addition to resistance to degradation, there are a number of other properties of pesticides which determine their behavior and fate. One is how volatile they are; that is, how easily they evaporate. The ones that are most volatile have the greatest potential to go into the atmosphere and, if persistent, to move long distances. Another important property is solubility in water; or how easily they dissolve in water. If a pesticide is very soluble in water, it is more easily carried off with rainwater, as runoff or through the soil as a potential groundwater contaminant (leaching). In addition, the water-soluble pesticide is more likely to stay mixed in the surface water where it can have adverse effects on fish and other organisms (Sapozhnikova et al., 2004). Pesticides movements in the hydrologic cycle is schematically presented in Figure 2. If the pesticide is very insoluble in water, it usually tends to stick to soil and also settle to the bottoms of bodies of surface water, making it less available to organisms.

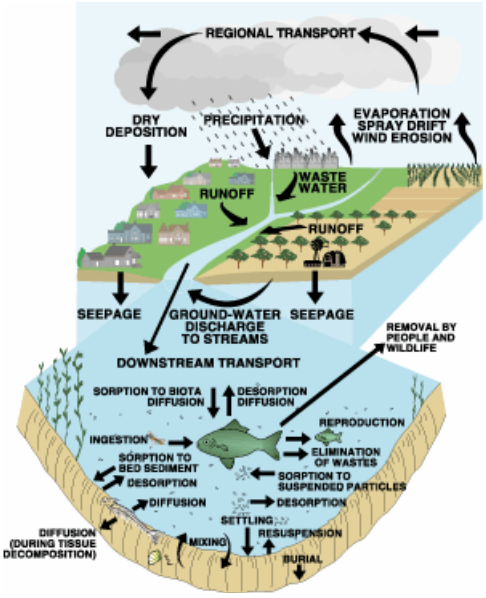


Figure 2. Pesticide movement in the hydrologic cycle including pesticide movement to and from sediment and aquatic biota within the stream. (Modified from Majewski and Capel, 1995).

From a knowledge of these and other characteristics, it is possible to predict in a general sense how a pesticide will behave. Unfortunately, more precise prediction is not possible because the environment itself is very complex. There are, for example, huge numbers of soil types varying in the amount of sand, organic matter, metal content, acidity, etc. All of these soil characteristics influence the behavior of a pesticide so that a pesticide which might be anticipated to contaminate groundwater in one soil may not do the same in another. Similarly, surface waters vary in their properties, such as acidity, depth, temperature, clarity (suspended soil particles or biological organisms), flow rate and general chemistry. These properties and others can affect pesticide movement and fate. With such great complexity, scientists

cannot determine exactly what will happen to a particular pesticide once it has entered the environment. However, they can divide pesticides into general categories with regard to, for example, persistence and potential for groundwater contamination and they can also provide some idea as to where the released pesticide will most likely be found at its highest levels. Thus, it is possible to gather information which can help make informed decisions about what pesticides to use in which situations and what possible risks are being faced due to a particular use.

The use of chemicals which are highly toxic for humans and animals in agriculture necessarily requires correct and precise management of all the phases of treatment, from transportation and storage to preparation of the spray mixture and the final disposal of residues (Drummond, 1998; McAllan, 1998; Balsari and Marucco, 2001). In particular, points where possible sources of pollution may be concentrated include all operations in which chemicals are manipulated by operators. Generally speaking, these points of high pollution result from management methods unfriendly to the environment. They can thus expect to be reduced considerably once farmers are made aware of the problem and apply some improvements in their practices (Fait et al., 2004). While open water presents an immediate problem to health and aquatic organisms, groundwater contamination will persist for very long periods, and may affect future generations as well as current users. The pathway depends on the degree to which the chemical is inactivated in soil by undergoing a chemical transformation such as hydrolysis or sorption, or whether it combines with organic matter and clay in forms where the activity is not altered. In this case the pesticide will still affect sensitive organisms, even though now adhering to particles that can be transported by overland flow or through preferred pathways in the soil profile (Kennedy et al., 1995). Certain chemicals are regularly found in catchments where

pesticides are applied to bare-soil cropping systems, as the result of runoff events that occur unexpectedly shortly after spraying. Today, farmers are increasingly aware of the complex interrelationships between agricultural practices and environmental quality. Modern farmers now consider the timing of agricultural chemical application and irrigation, the amount and style of pesticide application, specific crop needs, and local weather conditions in their pesticide and fertilizer use. Pesticides applied in plant nurseries and greenhouses are less visible forms of agricultural chemicals than those applied to fields and orchards. But any form of chemical application can potentially contaminate water sources if not managed properly. Compared to herbicides, currently used insecticides were less frequently found in agricultural streams, and even less in groundwater underlying agricultural areas. This results from their relatively low application rates and rapid breakdown in the environment. In contrast, historically used insecticides still persist in agricultural streams because of their resistance to breakdown in the environment.

1.3 PAHs: Origin and Environmental Fate

Polycyclic Aromatic Hydrocarbons (*PAHs*) are chemical compounds that consist of fused aromatic rings and do not contain heteroatoms or carry substituents. They are primarily formed by incomplete combustion of carbon-containing fuels such as coal, oil, gas, wood, garbage, or other organic substances, such as tobacco and charbroiled meat (Muthumbi, 2003). The simplest PAHs, as defined by the International Union on Pure and Applied Chemistry (IUPAC), are phenanthrene and anthracene. Smaller molecules, such as benzene and naphthalene, are not formally PAHs, although they are

chemically related they are called one-ring (or mono-) and two-ring (or di-) aromatics. PAHs containing up to six fused aromatic rings are often known as "small" PAHs and those containing more than six aromatic rings are called "large" PAHs. PAHs of three rings or more have low solubility in water and a low vapor pressure. As molecular weight increases, aqueous solubility and vapor pressure decrease. The aqueous solubility decreases approximately one order of magnitude for each additional ring. Because of these properties, PAHs in the environment are found primarily in soil and sediment, as opposed to in water or air.

PAHs enter the environment mostly as releases to air from volcanoes (NRC, 1983), forest fires, residential wood burning, and exhaust from automobiles and trucks. They can also enter surface water through discharges from industrial plants and waste water treatment plants (Barrick, 1982) and they can be released to soils at hazardous waste sites if they escape from storage containers.

Natural crude oil and coal deposits contain significant amounts of PAHs, as do combustion products and smoke from naturally occurring forest fires. PAHs can occur in the air, either attached to dust particles or as solids in soil or sediment, and in particles suspended in water (Marvin et al., 2004).

The global movement of PAHs can be summarized as follows: PAHs released to the atmosphere are subject to short- and long-range transport and are removed by wet and dry deposition onto soil, water and vegetation. In surface water, PAHs can volatilize, photolyze, oxidize, biodegrade, bind to suspended particles or sediments, or accumulate in aquatic organisms (with bio-concentration factors often in the 10-10000 range). In sediments, PAHs can biodegrade or accumulate in aquatic organisms. PAHs in soil can volatilize, undergo abiotic degradation (photolysis and oxidation),

biodegrade, or accumulate in plants. PAHs in soil can also enter groundwater and be transported within an aquifer.

Transport and partitioning of PAHs in the environment are determined to a large extent by physicochemical properties such as water solubility, vapor pressure, Henry's law constant, octanol-water partition coefficient (K_{ow}), and organic carbon partition coefficient (K_{oc}). In general, PAHs have low water solubility. The Henry's law constant is the partition coefficient that expresses the ratio of the chemical's concentrations in air and water at equilibrium and is used as an indicator of a chemical's potential to volatilize. The K_{oc} indicates the chemical's potential to bind to organic carbon in soil and sediment. The K_{ow} is used to estimate the potential for an organic chemical to move from water into lipid and has been correlated with bio-concentration in aquatic organisms.

The PAH content of plants and animals living on the land or in water can be many times higher than the content of PAHs in soil or water. PAHs can break down to longer-lasting products by reacting with sunlight and other chemicals in the air, generally over a period of days to weeks. Breakdown in soil and water generally takes weeks to months and is caused primarily by the actions of microorganisms.

PAHs can be harmful to human health under some circumstances. Several of the PAHs, including benz[a]anthracene (Andrews et al., 1991; Cavalieri et al., 1991), benzo[a]pyrene (Rojas et al., 2004; Das et al., 2007), benzo[b]fluoranthene (Kameda et al., 2005), benzo[j]fluoranthene (Shimada et al., 2001), benzo[k]fluoranthene (Spink et al., 2007), chrysene (Lloyd and Hanawalt, 2002), dibenz[a,h]anthracene (Shimada et al., 2001), and indeno [1,2,3-c,d]pyrene (Brandt and Watson, 2003), have caused tumors in laboratory animals when they breathed these substances in the air, when they ate them, or when they had long periods of skin contact with them. Studies of

people show that individuals exposed by breathing or skin contact (Anderson et al., 1995; Schoket et al., 1988) for long periods to mixtures that contain PAHs and other compounds can also develop cancer.

1.4 Technologies Aimed at Removing Pesticides and PAHs from Waters

In Italy, over 75% of the drinking water comes from groundwater. This source appears every year more contaminated by pesticides and PAHs, which enter the environment through different ways, such as leaching, irrigation, percolation, transportation within aquifers and so on. Removal of organic pollutants from waters is performed using specific treatments and/or processes. The most important ones are *membrane filtration*, *adsorption*, *ozonization*, *oxidation* and *phytoremediation*.

1.4.1 Membrane Filtration

Membrane filtration is the process of separating mixtures of materials depending on the particle size. When used for polluted water treatment, membrane filtration processes offer a high level of purification and a low footprint and relatively low energy consumption. Pressure-driven techniques, such as reverse osmosis and nanofiltration, are the most effective filtration procedures.

Reverse osmosis is a separation process that uses pressure to force a solvent through a membrane that retains the solute on one side and allows the pure solvent to pass to the other side. More formally, it is the process of forcing a solvent from a region of high solute concentration through a membrane to a region of low solute concentration by applying a pressure in

excess of the osmotic pressure. The membrane used is semi permeable, meaning it allows the passage of solvent but not of solute. Observations showed that pesticide removal mostly depends on pesticide molecular weight and hydrophobicity. Membrane composition increased salt rejection property and pesticide removal performance (Bhattacharya et al., 2006; Kosutic et al., 2005; Sarkar et al., 2007).

Nanofiltration uses the same principle of the reverse osmosis, but applying lower pressures. In this way, it is possible to separate components having a dimension higher than 1000 Daltons. Because of the use of lower pressure, nanofiltration results to be cheaper than reverse osmosis. Nanofiltration membranes can remove hazardous organic micro-pollutants, such as pesticides and PAHs, adsorbing the pollutants at value >90%. The adsorption properties were controlled by both the hydrophobicity (n-octanol/water partition coefficient, logP) and the molecular shape of the solute. The solute permeability of a pesticide for a membrane can be expressed as a linear combination of the following two factors: adsorption property on the membranes, and molecular width of the solutes (Jung et al., 2005; Kiso et al., 2002; Nghiem and Schäfer, 2005; Van der Bruggen and Vandecasteele, 2003).

1.4.2 Adsorption

In the last years, many researchers evaluated the application of different matrices to remove pesticides and PAHs from water by adsorption. Active carbon (Ayranci and Hoda, 2005; Heijman et al., 2002; Matsui, et al., 2002), clays (Gonzalez-Pradaset al., 2003; Zeledon-Toruno et al., 2007; Chang, et al., 2004) and organoclays (Pal and Vanjara, 2001; Lemic et al., 2006) have been studied.

Active carbon**,** also called activated charcoal or activated coal, is a general term which covers carbon material mostly derived from charcoal. It possesses an extremely high surface area (500 m^2 g^{-1}) and a porous structure with macro, micro and meso pores that provide a high sorbing capacity. For this reason, it is used in gas purification, metal extraction, water purification, medicine, sewage treatment, air filters in gas masks and filter masks, filters in compressed air and many other applications.

Clays and organoclays are widely used in a wide range of applications as a result of their high cation exchange capacity, swelling capacity, high surface areas and consequential strong adsorption capacities. They containing appropriate functional groups can be a useful strategy for the removal of organic pollutants from the environment. Most organoclays displayed higher affinity for the pesticides than the untreated clay, but the improvement in sorption capacity varied with the characteristics of the pesticide and the interlayer organic cation.

Recently nanoparticles in solution state and supported over activated alumina are proposed for removal of pesticides in surface waters (Nair and Pradeep, 2007).

1.4.3 Ozonization

Ozone (O_3) was discovered in 1839 by Christian Friedrich Schonbein as a by-product of the oxygen (O_2) generated at the anode during electrolysis of sulphuric acid (Schonbein, 1839). Ozone is an allotropic version of oxygen with three atoms, and is, after fluorine, the second most powerful oxidant and disinfectant which can be used technically. It is a gas with an oxidation potential of 2.07 eV and is up to 20 times more effective than chlorine. It has been used successfully for more than 100 years for the treatment of drinking

water and industrial water. Ozone is an environmentally benign gas which can be produced in the required quantities close to the point of use and quickly breaks down into normal oxygen. In contrast to most other oxidants and disinfectants, ozone generates no undesirable or toxic by-products. Ozone oxidation power has been studied with pesticides (Orlandini et al., 1996; Suty et al., 2004) and PAHs (Bernal-Martinez et al., 2007; Kornmuller et al., 1997). Ozonization seems to be even more effective if coupled with an active carbon filter able to remove the organic matter remaining after oxidation (Orlandini et al., 1996).

1.4.4 Oxidation

Advanced Oxidation Processes (AOPs), including ultraviolet (UV) radiation, ozone, and/or hydrogen peroxide, are used to destroy organic contaminants in water. UV oxidation is a destruction process that oxidizes organic constituents in polluted water by the addition of strong oxidizers and irradiation with UV light. Oxidation of target contaminants is caused by direct reaction with the oxidizers, UV photolysis, and through the synergistic action of UV light, in combination with ozone (O_3) and/or hydrogen peroxide (H_2O_2). If complete mineralization is achieved, the final products of oxidation are carbon dioxide (CO_2), water, and salts. The main advantage of UV oxidation is that it is a destruction process, as opposed to air stripping or carbon adsorption, for which contaminants are extracted and concentrated in a separate phase. UV oxidation processes can be configured in batch or continuous flow modes, depending on the throughput under consideration. Practically any organic contaminant that is reactive with the hydroxyl radical can potentially be treated.

Oxidation is based on the hydroxyl radical formation, through Fenton reaction using H_2O_2 or through photocatalytic processes with titanium dioxide (TiO_2). The Fenton process is an advanced oxidation process to degrade organic pollutants that are poorly biodegradable. This process can potentially be integrated into an existing water treatment process to enhance organic compounds removal. It can operate at low concentrations of contaminant and can often completely mineralize the compound or convert it into a less toxic form (Kruithof et al., 2002; Gutierrez et al., 2007; Derbalah et al., 2004; Shemer and Linden, 2007). Photocatalytic oxidation over titanium dioxide is a "green", sustainable process for the treatment and purification of water and wastewater (Li Puma et al., 2004).

1.4.5 Phytoremediation

Phytoremediation consists in decontaminating polluted soils, water or air with plants able to contain, degrade or eliminate metals, pesticides, solvents, explosives, crude oil and its derivatives, and various other contaminants, from the mediums that contain them (Singh et al., 2002). It is clean, efficient, inexpensive and non-environmentally disruptive. A range of processes mediated by plants are useful in treating environmental problems:

- *Phytoextraction* - uptake and concentration of substances from the environment into the plant biomass.
- *Phytostabilization* - reducing the mobility of substances in the environment, for example by limiting the leaching of substances from the soil.
- *Phytotransformation* - chemical modification of environmental substances as a direct result of plant metabolism, often resulting in

their inactivation, degradation (phytodegradation) or immobilization (phytostabilization).

- *Phytostimulation* - enhancement of soil microbial activity for the degradation of contaminants, typically by organisms that associate with roots. This process is also known as *rhizosphere degradation*.
- *Phytovolatilization* - removal of substances from soil or water with release into the air, sometimes as a result of phytotransformation to more volatile and / or less polluting substances.
- *Rhizofiltration* - filtering water through a mass of roots to remove toxic substances or excess nutrients. The pollutants remain absorbed in or adsorbed to the roots.

1.5 Bio-sorbents to Remove Toxic Substances: Agricultural and Food Industries By-products for Biobeds Preparation

A potential method for pesticides cleaning from water is the use of biological reactors, such as *biobed* or *biomassbed*. Biobeds and biomassbeds are classed as *waste recovery systems*. Biobed is a lined structure filled with a *biomix*. The organic filter or 'biomix' consists of a mixture of straw (wheat or barley), compost (preferably peat free) and topsoil. The three parts are mixed following this proportion: 1 part of compost, 1 part of topsoil and 2 parts of straw. When runoff water contaminated with pesticides is directed through the biobed, pollutants stick to the organic material and 'clean' water is released. The retained pesticides are then degraded by micro-organisms, and the treated water can be re-used in irrigation or as the carrier for pre-crop total herbicide applications (Fogg et al., 2004a, 2004b, 2004c, Rose et al., 2004). If it is stored securely for one year, the biobed material can be land

spread on agricultural land as long as it provides agricultural benefit or ecological improvement. The concept of biobeds was initially developed in Sweden in the early 1990s and has since been widely adopted with over 1000 biobeds in use in Sweden (Fogg et al., 2004a). The Swedish biobed design was unlined and developed as a solution for dealing with accidental and often unobserved splashes and spills in the pesticide handling area and was not used to treat sprayer washings. Currently, biobeds are also being tested in Denmark, Norway, France, Belgium and England.

There are two main biobed systems. A lined indirect biobed where all filling, mixing and sprayer washdown happens on an impermeable, bounded pesticide handling area. All liquid is diverted via a sealed drainage system to a nearby biobed. The second biobed system is the lined direct biobed. All filling, mixing and sprayer washdown happens directly above the biobed with access provided by a metal grid and support frame. All liquid falls directly onto the biobed.

An adapted biobed to the Italian conditions is the biomassbed, schematically shown in Figure 3. The biomass used is a mixture of material coming from the farm: topsoil, green compost and chopped vine-branches coming from winter pruning (Vischetti, et al., 2004). Studies related with the decontamination of polluted waters by cymoxanil, chorpyriphos, cyprodinil, fenitrothion, fludioxonil, flufenoxuron, iprovalicarb, metalaxil and penconazolchlorpyrifos, metalaxyl and imazamox showed that the half-life of all pesticides in the reactors was shorter than the one observed in the soil (Vischetti, et al., 2007; Coppola et al., 2007; Fait et al. 2007).

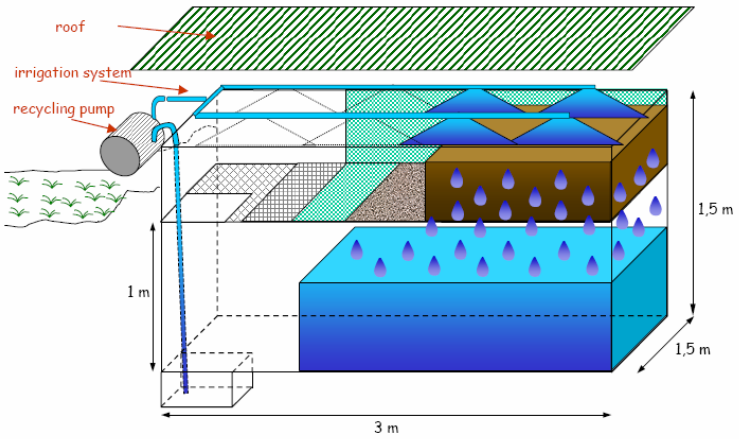


Figure 3. Schematic representation of a biomassbed.

1.6 Olive Oil Industry By-products: Production, Environmental Problems and Regulation

The olive oil is greatly important in our culture, with over 750 million of olive trees cultivated worldwide and about 95% of those trees in the Mediterranean region. Nearby 93% of the global production comes from European Union and 93% of the European production comes from Spain, Italy, Turkey and Greece. Spain alone accounts for more than 30% of the world production, which was 2.6 million metric tons in 2002. In 2006 Turkey accounted for over 25% of the global market (USDA, 2005).

On the other hand, the olive oil production generates important amounts of industrial wastes and by-products which can be described as follows (Archer, 1997):

- **Crude Olive Cake** - The residue which remains after the first pressing of the olives through traditional and continuous machines. There is still a small amount of oil in this cake.
- **Exhausted Olive Cake** - The residue that is left after the above crude olive cake has any remaining oil extracted from it by using solvents such as hexane.
- **Partly Destoned Olive Cake** - Produced if some of the crushed olive seeds are removed from the paste after processing.
- **Olive Pulp** - The residual paste which is produced if the whole olive seeds are removed from the paste prior to processing.
- **Vegetable Water** - The brown watery liquid which has been separated from the oil by centrifugation or sedimentation after pressing.

Olive oil production can be performed in two different ways, by a 2-phase and a 3-phase process. The main difference between the 2- and 3-phase processes is that the former uses hardly any water during the oil extraction and the latter, a significantly larger volume of water. Two processes lead to differences in the general physical characteristics of olive husk waste generated. The 2-phase process waste has a consistency of thick paste, dries to hard solid, low porosity and small particle size. The 3-phase waste has a not pasty consistency, hardens into loose solid, higher porosity and larger particle size.

Olive oil mill wastes can be used like other amendments to improve soil fertility and modify the soil physical and chemical properties of semiarid Mediterranean soils (Cox et al., 1997; Riffaldi et al., 1993; Abu-Zreig and Al-Widyan, 2002; Pagliai et al., 2004). It has been shown that agricultural wastes of the olive production can be used without further treatment (Brunetti et al., 2005). In particular, solid olive mill waste applied to soil resulted in an

increase of simazine and imazaquin retention, with a positive effect of reducing herbicide leaching into groundwater (Cox et al., 1997; Albarràn et al., 2003; Undabeytia et al., 2004). However, olive mill waste may have a high electrical conductivity and ammonia concentration (Francou et al., 2005), and it contains potentially antimicrobial compounds, such as polyphenols, and phytotoxic substances, such as organic acids (Capasso et al., 1992; Capasso et al., 1995; DellaGreca et al., 2004). Consequently, if applied as received, olive mill waste could have a negative environmental impact.

For this reason, the Italian Government in 1996 promulgated a regulation about olive waste disposal and/or recycle, the law 574/96: "New regulation about the agronomical use of olive mill waste". This law permits the agronomical utilization of olive mill waste upon approval of the local Authority.

Ten articles state the possibility of spreading the olive wastewaters as fertilizers on croplands. The wet solid waste from the two-phase process is classified as "soil conditioner" in derogation of the law 748/84 ("New regulation for fertilizers") and following integration and modifications (art.1). The maximum amount of waste to spread is 50 or 80 m^3 per ha, respectively for pressing process or in continuous process (art.2).

30 days before spreading, communication must be notified to the local Mayor and it must be supported by a technical report about soil and hydrological conditions, spreading system and time (art.3).

Spreading must be performed with a uniform distribution avoiding danger to water resources and living organisms (art.4).

Some lands are excluded from spreading: when the distance is less than 200 m from the built up areas or 300 m from the ground water draining areas,

when soil is used for growing vegetables and when soil has a water table depth of less than 10 m (art.5).
The maximum time of wastewaters storage is 30 days (Art.6).
Regional planning for wastewater spreading can be promulgated on the basis of local soil situation (art.7).
Administrative sanctions are provided for who violates the law (art.8).
Reports about the law application and the environmental state must be submitted to Parliament every 3 years (art.9).
General dispositions are present in the art.10 (Gazzetta Ufficiale, 1996).
Afterward a ministerial decree, D.L. July 2005, better defined some articles of the law 574/96. Spreading was forbidden during rainy time and the storage of waste waters was permitted in proofed containers for a longer time.
Therefore, Italy is the only olive oil producing Country with a special law for the disposal and/or recycle of olive processing wastes.

1.7 Current Technologies Aimed at Recycling and/or Disposing of Olive Mill Wastewaters

Olive mill wastewaters are a worldwide concern. Every olive oil producing Country try to cope with OMW disposal problem by using different strategies. The conventional methodologies used to dispose of OMW are listed below, with their advantages and disadvantages:

1- Evaporation lagoons

This consists in the storage of olive oil wastewaters in impermeable lagoons followed by liquid evaporation during summer season or with evaporation panels. Accumulated solid residues are removed before every season labor

and are used as fertilizers. This is a simple and rather cheap technique, however, it demands large areas, it can produce bad smell, attract insects and it is needed a long period of time for the treatment of the effluent.

2 – Mixed treatment with other effluents - biological process

This treatment is based on the addition of slow amounts of OMW to other organic effluents. This procedure consists on anaerobic digestion, and the final residue could be used as fertilizer. This system is technically complex and with high costs of implementation.

3 – Physical-chemical treatments

This process use flocculants and coagulants agents, and by enzymatic process, could be obtained a product to be used as liquid fertilizer. This methodology has low cost but also a low efficiency.

4 – Irrigation

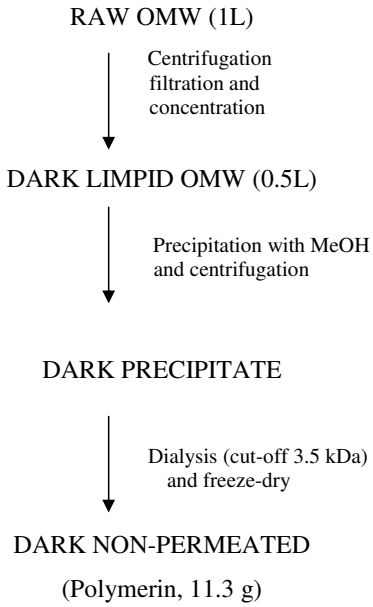
Olive oil wastewaters are concentrated in lagoons, and after pH correction, it can be used for irrigation. This is a rather cheap process, it can fertilize the soils, however, it can also cause contamination of the soils by phenols.

Nowadays, more and more researchers are tackling this issue, developing new technologies aimed at increasing the value of OMW or making the old methodologies cheaper and more effective. For example, Nair et al. (2007) proposed a composting process combining an anaerobic step and then a step of aeration with further addition of microbial mix. They demonstrated that this process was relatively short and the resulted compost was non-phytotoxic. A biological treatment was also proposed by Duarte et al. (2007), who developed the Jet Loop Reactor, a system able to remove 90% of COD

and 80% of phenolic compounds due to a combination of efficient oxygen transfer and a high turbulent mixing. Olive mill residues composting, which permit the degradation of toxic compounds, was suggested by different researchers who also proposed to re-use the cured compost in agriculture as eco-compatible and good quality organic amendments and fertilizers (Tomati et al., 1996; Cegarra et al., 1996; Ranalli et al., 2002). In addition, composts from different vegetal origin, due to their beneficial effects, were also suggested to be used as alternative means to control plant pathogens (Lazarovits, 2001; El-Masry et al., 2002; Raviv, 2005). Altieri and Esposito (2007) developed a technology named M.A.T.Re.F.O. [patent n. RM2004A000084] for recycling all kinds of raw OMW in agriculture. The procedure occurred at milling level by mixing OMW, previously destoned, with raw hygroscopic bulking agents (straw, wool waste, sawdust, olive leaves, twigs and pruning) in order to obtain a dryer non percolating and non bad-smelling mixture which was directly packaged into 20-30 kg net bags, hence resulting easy to be managed. Chemical properties of OMWM, recorded after short aerobic storage trials, make it suitable for different agronomic applications as soil amendment/fertilizer or as peat substitute in making nursery growth media or for mushroom cultivation. Other groups studied the earthworms capability to biodegrade OMW, finding, during the vermicomposting, a decrease of total organic matter content and water-soluble fraction (Benitez et al., 2002; Delgado-Moreno et al., 2007). Dephenolization and detoxification of olive wastes was also performed by using different kinds of fungi (Linares et al., 2003; Sampedro et al., 2005). Physical pre-treatments of olive mill wastewater also decreased considerably its phytotoxicity (Ginos et al., 2006). Many researchers are focusing their works on OMW disposal problem, but to date none of these approaches appears to offer universal solution (Cermola et al., 2004).

1.8 Polymerin: Recovery and Its Application in Environmental Technologies

Olive mill wastewater (OMW), as previously said, is a vegetable biomass wastes produced in a very limited period of the year, presenting disposal problems for its polluting properties, which are documented by high chemical oxygen demand (COD) and biological oxygen demand (BOD) (Demicheli and Bontoux, 1997; Arienzo and Capasso, 2000). Polluting properties are mainly due to their polyphenol content and its synergy with other naturally occurring compounds (Capasso, 1997; Capasso et al. 2002b) and make OMW unsuitable for discharge to soil and waters.



Scheme 1. Recovery of polymerin from olive mill wastewaters.

In order to obtain by-products to reuse in environmental technology processes and to reduce the organic polluting load of OMW, the recovery of organic matter from this waste was performed. This polymeric organic fraction was recovered from OMW by a three-step purification procedure (Scheme 1). In the first step 1L of OMW was centrifuged, filtered and concentrated to 0.5 L. In the second step, the concentrated sample was precipitated with cold methanol and centrifuged. In the third step, the dark precipitate was re-dissolved in ultra pure water, dialyzed and the non-permeated fraction was freeze-dried, giving a brown residue named *polymerin* (Capasso et al., 2002a).

Chemical analysis revealed polymerin was composed of carbohydrates, melanin and proteins (Table 2). The content of monosaccharides and amino acids, determined respectively by hydrolysis and AE-PAD (Anion Exchange with Pulsed Amperometric Detection) for carbohydrates, automated Amino Acid Analyzer for proteins, is reported in Tables 3 and 4

Table 2. Chemical composition of polymerin as reported by Capasso et al., 2002a.

Organic Composition	mg/100mg
Polysaccharides	47.4 (± 0,007)
Proteins	20.4 (± 0,034)
Melanin	30.2 (± 0,01)
Metals	2
Total	**100**
C	46.0 (± 0,205)
N	2.4 (± 0,007)
O + H	49.6

. Polymerin contained also metals, mainly K and, to a lesser extent, Na, Ca, Mg, Zn, Fe and Cu. Gel filtration chromatography of polymerin indicated that it was composed of a free polysaccharide and a supramolecular

aggregate of protein, melanin and polysaccharide, strongly attached to each other by a combination of covalent, hydrogen bonds and CH/π interactions.

Table 3. Monosaccharide composition of polymerin determined by HPAEC-PAD expressed in weight % as reported by Capasso et al., 2002a.

	% of sugar present in polymerin	**% of monosaccharides in the polysaccharide component**
Arabinose	20.9	38
Galactose	10.45	19
Glucose	9.9	18
Ramnose	5.5	10
Glucuronic acid	4.4	8
Galacturonic acid	3.85	7
Total	**55**	**100**

The metal component was bound to both the supramolecular aggregated portion and the free polysaccharide. The interaction occurred through carboxylate and other nucleophile functional groups, also in chelating form, identified in the acidic amino acids and uronic acids and in the melanin system (Capasso et al., 2002a). Successively, polymerin was also transformed into its metal derivatives, obtained by saturation of polymerin with various micronutrients (Cu, Zn, Mn, and Fe) and other metals of interest (Na and Al). Tests on tomato plants showed that only Mn derivative of polymerin caused witling of the plants, whereas all the other metal derivatives were non toxic (Capasso et al., 2002b). The humic acid nature of saturated polymerins and the presence of macro- and micronutrient metals (Cu, Zn, Fe, and Mn) suggest their use as bio-amendments and/or metal-biointegrators. In addition, the melanins are humic acid-type polymers which are involved in soil fertility (Paim et al., 1990; Biliska, 1996).

Table 4. Amino acidic composition of polymerin determined by automated Amino Acid Analyzer expressed in weight % as reported by Capasso et al., 2002a.

Amino acid	% of amino acids present in polymerin	Number of amino acids in the protein component
Asp+Asn	1.95	15
Glu+Gln	2.02	14
Cys	0.1	1
Thr	0.87	5
Ser	1.37	13
Pro	1.3	11
Gly	2.29	31
Ala	1.36	15
Val	1.35	12
Met	0.37	2
Ile	0.6	5
Leu	1.15	9
Tyr	0.55	3
Phe	0.54	3
Lys	0.054	1
His	0.088	1
Arg	0.64	4
Total	**16.6**	**143**

Besides, polymerin showed much lower COD and BOD values in comparison with those of raw OMW (Arienzo and Capasso, 2000). Finally, the polyelectrolyte characteristics of polymerin and its relatively high content of the easily removable K^+ suggest that this biomaterial may be used as a potential bio-filter for waters contaminated by heavy and/or unwanted metals. The use of vegetable biomasses for decontamination of wastewaters by adsorption of pollutants is of great importance and interest because it allows the resolution of both their disposal problem and the wastewater decontamination, by an environmentally clean process (Schneegurt et al., 2001).

The traditional techniques for the removal of metal ions from aqueous effluents are either not able to reduce concentrations to the levels required by law or prohibitively expensive (by activated carbon sorption or ion exchange process). Heavy metal originate from sources of pollution including agricultural fertilization, manufacturing processes, smelting and refining, refuse and wastewater treatment and fuel combustion. Bio-sorption technologies, in which vegetable biomass is used to accumulate heavy metals (Volesky and Holan, 1995; Aderhold et al., 1996), are methods that can replace conventional processes for remediating metal pollution in wastewaters, which require relatively expensive mineral adsorbents or flocculating agents (Reed and Nonavinakere, 1992). More specifically, biomaterials such as condensed tannin gel (Nakano et al., 2001), grape stalk (Foil et al., 2002) and sphagnum peat moss (Sharma and Forster, 1993) have shown interesting capacity for the removal of hexavalent chromium. Capasso et al. (2004) report a study on the treatment of wastewaters contaminated with Cr(III), Cu, and Zn by sorption on polymerin. Individual sorption showed that these metals were strongly sorbed on polymerin, following the order Cr(III) > Cu > Zn. Simulated wastewaters contaminated with Cu and Zn were purified after three sorption cycles by polymerin renewed at each cycle, whereas those containing a mixture of Cr(III), Cu, and Zn showed residues of Zn after five cycles. Polymerin sorbed poorly As(V) and Cr(VI). This latter was sorbed in form of Cr(III), produced by the redox reaction of Cr(VI) with polymerin (Sannino et al., 2006). Sorption of Zn, Cu, and Cr(III) on polymerin was higher than sorption on other bio-sorbents described in the literature (Volesky and Holan, 1995; Aderhold et al., 1996; Gardea-Torresday et al., 2001; Schneegurt et al., 2001; Wilson et al., 2003). In addition, the employment of polymerin in wastewater remediation processes should not only decontaminate the waters of heavy metals, but also enrich

them in nutrients such as Ca, Mg and K, which are released from polymerin during the clean-up process.

The reduction from Cr(VI) to Cr(III) indicate that from such a toxic waste it is possible to recover a useful metal of considerable industrial interest. As an example of a possible application in environmental technology processes, polymerin may be utilized to regenerate Cr(III) from Cr(VI) formed in the oxidation process of some tannery plants, before these wastes are discarded.

CHAPTER 2

Aims of the Thesis

The use of pesticides allows to protect crops and increase the production, but incorrect agronomical practices, leakage from equipment, incorrect disposal of waste and washings cause soil and surface waters pollution. In the last ten years, biological systems, named *biobeds*, as reported in the paragraph 5 of this chapter, were developed to remediate surface waters point-source pollution. Biobeds contain bio-filters (biomix) which are composed of soil, organic matter and straw (Fogg et al., 2003a, b and c; Henriksen et al., 2003). The organic matter of the biomix used to degrade pesticides was peat, compost and, recently, wine branch and citrus pulp (Vischetti et al., 2004). A complex bio-organic polymer, as polymerin, having a humic acid-like nature, and good sorbing capacity of anionic and cationic heavy metals, as previously mentioned, may be employed as bio-filter for the removal of ionic and ionizable pesticides from surface waters. Besides, polymerin may be utilized for the preparation of biobeds in the same farm where OMW are produced. The use of polymerin for remediation of pesticide-contaminated waters may represent a convenient method for bioremediation, because this material derives from a free cost agricultural waste and it has a low phytotoxicity and very low BOD and COD values, compared to raw OMW (Arienzo and Capasso, 2000). Therefore, its use may contribute to the resolution of OMW disposal problem, which is a very

polluting biomass. The sorbing capacity of metals by polymerin was very high as respect to other by-products used as metal bio-sorbents (Orhan and Buyukgungor, 1993; Volesky and Holan, 1995; Aderhold et al., 1996; Muraleedharan et al., 1991; Gardea-Torresday et al., 2001). In addition, the employment of polymerin in waste waters remediation processes may not only potentially decontaminate waters from ionic pesticides, but also enrich them in biologically useful metals as Ca, Mg and K, which are released from polymerin during the clean-up process such as occurred by the heavy metals as previously described.

Moreover, the application of polymerin to a larger range of pollutants, including not only ionic but also non-polar compounds, will make this bio-sorbent multitasking and, consequently, more useful and convenient.

Therefore, this thesis aim to achieve different objectives, dealing with the following topics:

1) Sorption studies on polymerin of model of ionic herbicides, such as paraquat and 2,4-D (chapter 3);
2) Study of wastewaters decontamination by using the sorption data previously obtained (point 1), for a potential application of polymerin as bio-filter in biobeds (chapter 3);
3) Sorption studies on polymerin of cyhalofop, a new generation and currently used herbicide (chapter 4);
4) Investigation about sorption on polymerin of non-polar compounds, such as phenanthrene (chapter 5);
5) Studies of the behavior of phenanthrene in the presence of polymerin, in a complex system consistent in polymerin interacting with aluminum oxides in micro- and nanoparticles (chapter 5).

CHAPTER 3

Sorption of Ionic Model Herbicides on Polymerin*

Abstract

This study investigated the sorption of paraquat and 2,4-D on polymerin, the humic acid-like fraction of olive mill wastewater. Effects of pH, contact time, initial concentration and sorbent dosage on the sorption of both herbicides were studied. Sorption mechanism of paraquat on polymerin was consistent with the ion exchange of this herbicide with Ca, Mg and K natively occurring in the sorbent; in contrast, 2,4-D was bound to polymerin by hydrogen bonding. Simulated wastewaters contaminated with paraquat were purified after three sorption cycles on polymerin renewed at each cycle, at a solid/liquid ratio of 0.5, whereas those containing 2,4-D showed a maximal residue removal of 44% after two sorption cycles at the same ratio. The possible application of this model to other water soluble herbicides, as well as the possible exploitation of polymerin as a bio-filter for the decontamination of pollution point sources is briefly discussed.

* A version of this chapter has been published.
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3.1 Introduction

The use of pesticides to protect crops is a current practice which permits a significant production increase. In association with this benefit, the risk of environmental pollution must be considered, and the soil and surface waters are mostly exposed. The quality of soil and surface waters deserves particular attention because of its importance in the ecosystem survival as well as in the supply of water for drinking and amenity use. Thus, their protection and, eventually, their remediation is a necessity of primary importance. In order to protect the environment and human health, it is important to develop methodologies to prevent pesticide contamination from point sources (Mason et al., 1999). Although environmental protection is well guaranteed by the European registration process for pesticides, there is a lack of legislative tools to control and prevent contamination by point sources. Recent research has demonstrated that only a small part of the applied pesticide reaches surface and ground waters through diffuse contamination via percolation, runoff, drainage and drift (Carter, 2000; Spanoghe et al., 2004; Guzzella et al., 2006). Contamination arising from sources such as non-approved use, poor practice, illegal operations, accidental releases from the farmyard and inputs of washings is increasingly recognized as contributing to water contamination (Mason et al., 1999; Carter, 1999; Kruger and Nilsson, 2001; Yoder et al., 2001).

Several workers have indicated that point sources (i.e. the spills and washings from the farmyard) can contribute between 18% and 84% of the pesticide load measured in individual catchments (Higginbotham et al., 1999; Muller et al., 2002). Better training of sprayer operators and good machinery maintenance can reduce the number of spills; releases to the farmyard due to spray tank washings could be minimized (Rose et al., 2001). The application of

treatment systems at the farm level could allow an improved protection of the aquatic ecosystem and a reduction of water treatment cost.

The possibility of using vegetable biomass wastes as a biofilter for the remediation of wastewaters generated at point sources was first described by Pussemier et al. (1998). Production of olive oil is a significant agricultural activity with great economic importance particularly in Mediterranean countries. However, it generates high amounts of wastewaters deriving from the olive mill process (olive mill wastewater or OMW). This waste causes disposal problems because of its highly polluting properties, which are characterized by high chemical oxygen demand (COD) and biological oxygen demand (BOD) (Arienzo and Capasso, 2000). Nevertheless, OMW may also be regarded as a free source of valuable vegetable biomass which recovery and utilization (Capasso et al., 2004) avoids the negative environmental impact associated with its discharge. The use of vegetal biomass with high sorption capacity for remediation of waters contaminated with pesticides or metals has been widely described in the literature over the last 10 years (Schneegurt et al., 2001). In particular, the utilization of the polymeric organic fraction of OMW named polymerin (Capasso et al., 2002) for remediation of contaminated waters by pesticides could represent a convenient method of bioremediation, because this material derives from a cost-free agricultural waste and possesses humic acid-like characteristics. The wide versatility of this kind of substances for the sorption of pesticides is relatively well documented (Hesketh et al., 1996; Celi et al., 1997; Akhtar et al., 2007). Previously, we demonstrated that polymerin exhibits strong sorption capacity for cationic heavy metals (Capasso et al., 2004) and, more recently, also for anionic heavy metals (Sannino et al., 2006). These findings suggest the potential for sorption of ionic or ionizable pesticides.

The main objective of this study was to investigate the sorption capacity of polymerin as a potential bio-filter to decontaminate waters polluted by ionic or

ionizable pesticides in point sources. To this end, paraquat and 2,4-D were selected as model compounds. Paraquat (also known as methyl viologen) is a widely used quaternary ammonium herbicide due to its excellent action within plant cells. There have been many authenticated cases of the detection of this cationic pesticide in water sources (Ritter et al., 2002) and the compound is highly toxic if deliberately or accidentally ingested (Chen and Lua, 2000). 2,4-D is a dichlorophenoxy acid which acts as a selective systemic herbicide. The compound is widely used for the control of broad-leaved weeds in cereals and other crops and is a frequently detected contaminant of ground- and surface waters (Gonzalez-Toledo et al., 2001). 2,4-D is currently being considered for inclusion onto an Environment Red List among chemical compounds which must be controlled to prevent water pollution (IPCS, 2002).

In this work, studies were carried out on (i) the sorption of paraquat and 2,4-D on polymerin and (ii) the remediation process of simulated wastewaters individually contaminated with these herbicides by cyclic sorption on polymerin renewed at each cycle. The potential environmental advantages deriving from the use of this bio-sorbent as a bio-filter for the remediation of water contaminated with soluble pesticides in the point sources is also discussed.

3.2 Experimental Section

3.2.1 Preparation and Characterization of Polymerin

Aliquots of 1 L of raw OMW were first centrifuged at 11500 *rpm* for 30 min and filtered through membranes of 1.2 and 0.2 μm (Millipore), obtaining a clear, dark solution, which was then concentrated to 0.5 L under reduced pressure. The solution thus obtained was treated with 1.5 L of cold methanol (-20 °C), and after 4 h a dark precipitate was obtained. This was separated by centrifugation at 11500 *rpm* for 30 min and re-dissolved in a minimum volume of high quality water, then dialyzed using membranes (Spectrum Medical Industry, Houston, TX) with molecular weight cut-off of 3.5 kDa, against 5 sequential volumes (1:5) per time, at 4-h intervals. The non-permeated dialyzed fraction was freeze-dried, leaving a black residue of 11.3 g, named polymerin (Scheme 1, Chapter 1) (Capasso et al., 2002).

3.2.2 Chemicals

2,4-D [(2,4-dichorophenoxy)acetic acid] and paraquat dichloride [1,1'-dimethyl-4,4'-bipyridinium] were purchased from Sigma-Aldrich Chemical Company (Poole, Dorset, UK; both 99.0% purity). All the solvents were of HPLC grade (Carlo Erba, Milan, Italy) and were used without further purification. All the other chemicals were obtained from Sigma-Aldrich unless otherwise specified.

3.2.3 Chemical Analysis

The carbon elemental analysis was performed using a Fisons EA 1108 elemental analyzer for hydrogen, carbon, nitrogen and sulfur. The metal

composition was determined by a Perkin–Elmer model 3030 B atomic absorption spectrometer equipped with deuterium-arc background correction. An
air–acetylene flame was used as the atomization source. Stock standard solutions of each metal cation (1 $g \cdot L^{-1}$) were obtained from BDH Reagents (Poole, UK). The average and standard deviation of three absorption measurements were recorded for each sample.

3.2.4 Diffuse Reflectance Infrared Fourier Transform (DRIFT) Spectroscopy Analysis

DRIFT spectra were obtained using a Perkin–Elmer Spectrum One FT-IR with a resolution of 1 cm^{-1}. Sample preparation was performed as follows: 0.2 mg of sample was mixed with 200 mg of KBr (FT-IR grade, Aldrich, Chemical Co., Milwaukee, WI). The mixture was finely ground in an agate mortar and transferred to a sample holder. Its surface was smoothed with a microscope glass slide, and DRIFT spectra were recorded.

3.2.5 Estimation of the Point of Zero Charge (pzc) by Potentiometric Titration

Dry samples (250 mg) of raw polymerin were dissolved in 100 mL of electrolyte solution (0.1, 0.01 and 0.001 $mol \cdot L^{-1}$ NaCl), brought to pH 9.0 with 2.0 $mol \cdot L^{-1}$ NaOH and successively brought to pH 2.0 with 0.05 $mol \cdot L^{-1}$ HCl by an automatic titrator (VIT 909 Videotritator, Copenhagen) under nitrogen stream. The pzc of polymerin was determined from a potentiometric titration curve as a function of pH at different ionic strength (Noh and Schwarz, 1989; Tsai et al., 2003).

3.2.6 Sorption Experiments

3.2.6.1 Sorption Methodology

Stock solutions of pesticide were prepared by dissolving 100 mg of paraquat in 100 mL of distilled water (final concentration 3.88 mmol·L^{-1}), and 50 mg of 2,4-D in 100 mL of 0.03M KCl (final concentration 2.26 mmol·L^{-1}), respectively. These solutions were then kept refrigerated. All the experiments were conducted at different solid/liquid ratios. Ratios of 0.5, 1.25 and 2.5 were generated by adding 10, 25 and 50 mg, respectively, of polymerin previously dried at 100±1°C for 1h, to a final volume of 20 mL at 20±1°C. The pH was controlled by addition of 0.01 mmol·L^{-1} HCl or KOH to the suspension. Preliminary experiments were conducted to select an experimental methodology suitable for the separation of polymerin from the suspension. After incubation, the sorbent was separated from the suspension using three different systems. In the first, the suspension was transferred into an ultrafiltration cell equipped with a magnetic stirrer and a 1000 Da cut-off membrane. The cell was placed under a nitrogen flow at a pressure of 3 bar. In the second, the suspension was filtered by centrifugation at 3500 rpm for 50 min, using an Amicon Centricon system (Millipore) having a horizontal membrane with a cut-off of 3000 Da. In the third, the suspension was filtered by centrifugation at 5500 rpm for 20 min, using an Amicon Ultra system (Millipore) having two transversal membranes with cut-off of 5000 Da. The ultrafiltration cell showed: (i) a significant retention of both pesticides (6% of paraquat and 23% of 2,4-D); (ii) a great consumption of nitrogen, and (iii) a slowdown of the filtration and, consequently, of the entire experimental procedure. In contrast, the Amicon Centricon system displayed a lesser retention of both pesticides (0% of paraquat and 7% of 2,4-D); it made the process more rapid and did not require the use of

nitrogen. Its only disadvantage arose because each system could only be reused twice. The Amicon Ultra system, in addition to the advantages reported for the Amicon Centricon system, showed lower retention of both pesticides (0% of paraquat and 4% of 2,4-D) and it could be reused up to twenty times. The three different separation systems showed that even if they allowed a satisfactory sorbent separation, they interfered significantly with the analytical determination of the pesticides because of the retention processes. Therefore, all the experiments were performed using the Amicon Ultra system. The amount of paraquat and 2,4-D sorbed on polymerin was calculated as the difference between the initial concentration of pesticide and the amount presents in the equilibrium solution.

3.2.6.2 *Analytical Determination of the Two Herbicides*

Paraquat was determined using a Perkin–Elmer Lambda 25 UV/Vis Spectrometer at a wavelength of 257 nm. 2,4-D was analyzed with a Perkin–Elmer 410 HPLC equipped with an LC 90 variable wavelength UV-visible spectrophotometric detector and Perkin Elmer Network Chromatography Interface 901/902 with Turbochrom Pro 4.x. A Macharey-Nägel Nucleosil 100-5 C18 column (stainless steel 250 x 4 mm^2). The mobile phase, comprising a binary system of 40:60 acetonitrile:phosphate buffer (0.04 M, pH 2.95) (B), was pumped at 1.2 mL min^{-1} flow in an isocratic mode. The detector was set at 283 nm. Injection volume was 20 µL. The quantitative determination of paraquat and 2,4-D was performed elaborating their corresponding calibration curves, 0.01–0.04 and 0.2 x 10^{-3}–0.7 mmol·L^{-1}, respectively. Polymerin blanks and paraquat or 2,4-D controls were subjected to the same conditions as samples.

3.2.6.3 Effect of pH

Experiments were carried out by adding a fixed pesticide concentration (0.27 and 0.22 mmol·L^{-1} for paraquat and 2,4-D, respectively) and varying the pH (from 3.0 to 8.0 for paraquat and from 2.0 to 5.0 for 2,4-D). The suspensions were shaken for 24 h. The pesticides were separated from the sorbent and analyzed as described above.

3.2.6.4 Effect of Time

Experiments were performed using 0.27 mmol·L^{-1} of paraquat at pH 5.7 (native pH of polymerin) and 0.22 mmol·L^{-1} of 2,4-D at pH 3.0, respectively. The suspensions were stirred for 0.25, 0.5, 1, 2, 4, 6, 8, 24, 30 and 48h for both pesticides. The data were analyzed using the Lagergren equation (Namasivayam and Yamuna, 1995):

$$\log(q_e - q) = \log q_e - K_a t / 2.303 \qquad (1)$$

where q_e and q are the amount of sorbed pesticide (mmol·kg^{-1}) at equilibrium and at time t, respectively, K_a is the rate constant of sorption (min^{-1}) and t is time (min).

3.2.6.5 Sorption Isotherms

Samples containing variable concentrations of pesticide (from 0.1 to 4.0 and from 0.1 to 1.0 mmol·L^{-1} for paraquat and 2,4-D, respectively) at pH 5.7 for paraquat and pH 3.0 for 2,4-D were incubated at different solid/liquid ratios. They were shaken for 4 and 24h for paraquat and 2,4-D, respectively. The

sorption data were analyzed according to the Langmuir and Freundlich equations.

The Langmuir equation can be written as follows:

$$S = S_m KC_e /(1 + KC_e) \quad (2)$$

where S is the amount of pesticide sorbed on polymerin (mmol·kg^{-1}), K is the Langmuir constant related to the binding energy, S_m is the maximum amount of pesticide sorbed (mmol·kg^{-1}) and Ce is the equilibrium concentration of pesticide (mmol·L^{-1}), according to Giles et al. (1974).

The Freundlich equation is:

$$S = K_f C_e^{\ N} \quad (3)$$

where S is the pesticide sorbed by polymerin (mmol·kg^{-1}) and Ce represents the equilibrium concentration of the pesticides (mmol·L^{-1}). K_f [(mmol·kg^{-1})/(mmol·L^{-1})^N] and N (dimensionless) are the Freundlich sorption parameters.

The single point sorption coefficient (K_d) was used widely for comparisons in the literature due to isotherm nonlinearity (Wang et al., 2006). Therefore, the single point K_d was calculated based on Freundlich equation at a given concentration (Ci).

$$K_d = S / C_i = K_f \times C_i^{\ n-1} \quad (4)$$

3.2.7 Determination of the Native Metals Released from Polymerin

A 10 mg aliquot of polymerin, previously dried at 100±1°C for 1h, was equilibrated at 20±1°C with 18 mL of ultrapure water for paraquat and 16.5 mL of 0.03M KCl for 2,4-D. Predetermined amounts of pesticide stock solutions were pipetted into the flasks to give 0.40 $mmol \cdot L^{-1}$ of paraquat or 2,4-D. The final suspensions (20 mL) were shaken for 4 or 24h, for paraquat and 2,4-D respectively, and their pH values were adjusted periodically to the optimum pH of each pesticide.

3.2.8 Cyclic Sorption on Polymerin

Cyclic experiments for sorption of paraquat and 2,4-D were carried out at three different ratios. In the first cycle, 800 $mmol \cdot kg^{-1}$ of paraquat, corresponding to 0.38, 0.97 and 1.94 $mmol \cdot L^{-1}$, were added to the polymerin suspensions in the presence of 10, 25 and 50 mg of polymerin, respectively. Similarly, 250 $mmol \cdot kg^{-1}$ of 2,4-D, corresponding to 0.12, 0.32 and 0.65 $mmol \cdot L^{-1}$, were added for each quantity of sorbent used, as reported above. Samples containing paraquat or 2,4-D with a final volume of 20 mL were shaken for 4 and 24 h, respectively. After the contact time, the sorbent was separated from the suspension, the filtrate was analyzed and the amount of sorbed pesticide was determined. For the second cycle, the filtrate was made up to 20 mL before adding fresh polymerin (10, 25 and 50 mg). The experiments were repeated until the equilibrium concentrations remained constant.

3.2.9 Analysis of the Data

All the experiments were performed in triplicate and the relative standard deviation was lower than 4%.

3.3 Results and Discussion

3.3.1 Characterization of the Sorbent

Polymerin was recovered from OMW and characterized chemically and spectroscopically (DRIFT) according to the procedure described by Capasso et al. (2002). The material was confirmed to be a humic acid-like polyelectrolyte, consisting of carbohydrates, melanin and proteins. It also contains metals such as Ca, Mg, K, Na and Fe (III), that are naturally bound and chelated through carboxylate anions and other characteristic nucleophilic functional groups present in polymerin. The chemical and physico-chemical properties of polymerin are reported in Table 1.

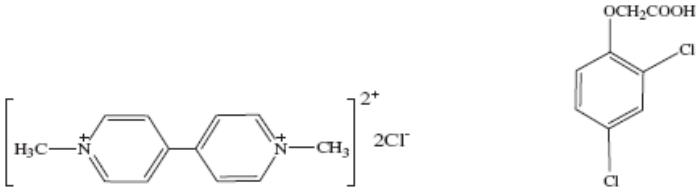
TABLE 1. Chemical Properties of Polymerin

	polysaccharide (%, w:w)	**protein (%, w:w)**	**melanin (%, w:w)**	**metals[a] (%, w:w)**	**rmw (Da)**	**carbon (%, w:w)**
polymerin	43.07	22.40	29.76	4.77 [Na (0.91), K (1.59), Ca (1.15), Mg (0.41), Fe (0.71)]	first peak: 3500<rmw<10000 second peak: 45000	41.49

[a]The values convertered to meq·kg^{-1} are: 3343 [Na (397), K (405), Ca (1630), Mg (570), Fe (341)].

3.3.2 Sorption Studies

Preliminary experiments were conducted to select the most suitable experimental conditions for the sorption of paraquat and 2,4-D (Figure 1) on polymerin.



Paraquat dichloride [1,1'-dimethyl-4,4'-bipyridinium] 2,4-D [(2,4-dichlorophenoxy)acetic acid]

Figure 1. Structural formulae of paraquat and 2,4-D.

Kinetic runs carried out at native pH (5.7) by adding 0.27 mmol·L^{-1} of paraquat or 0.22 mmol·L^{-1} of 2,4-D, respectively, showed paraquat to be significantly sorbed after 4h of incubation, whereas 2,4-D was scarcely sorbed after an incubation time of 24h. This behavior was observed at all selected solid/liquid ratios (0.5, 1.25 and 2.5) (data not shown). Sorption experiments for the two herbicides were carried out as a function of pH and are reported in Figures. 2a and b, respectively. The sorbed amount of paraquat, for each solid/liquid ratio, was insignificant at low pH (pH = 3.0), increased rapidly up to the native pH of polymerin (5.7) and then decreased between pH 6.0 and 7.0. This decrease was observed in the experiments performed at solid/liquid ratios of 0.5 and 1.25, whereas, sorption above pH 5.7 was fairly stable in the presence of 50 mg of polymerin. In contrast, a measurable sorption of 2,4-D was observed at acidic pHs (2.0–3.0), while by increasing pH, a considerable reduction in the sorbed 2,4-D was found. Greatest sorption of both pesticides occurred at a solid/liquid ratio of 0.5 (260 and 140 mmol·kg^{-1}, for paraquat and 2,4-D,

respectively). The optimum pH for sorption was 5.7 for paraquat (coincident with the native pH of polymerin) (Figure 2a). Sorption of 2,4-D (Figure 2b) was slightly greater at pH 2.0, but the suspension showed significant turbidity at this pH, most likely due to the formation of aggregates. Thus the sorption experiments were conducted at pH 3.0.

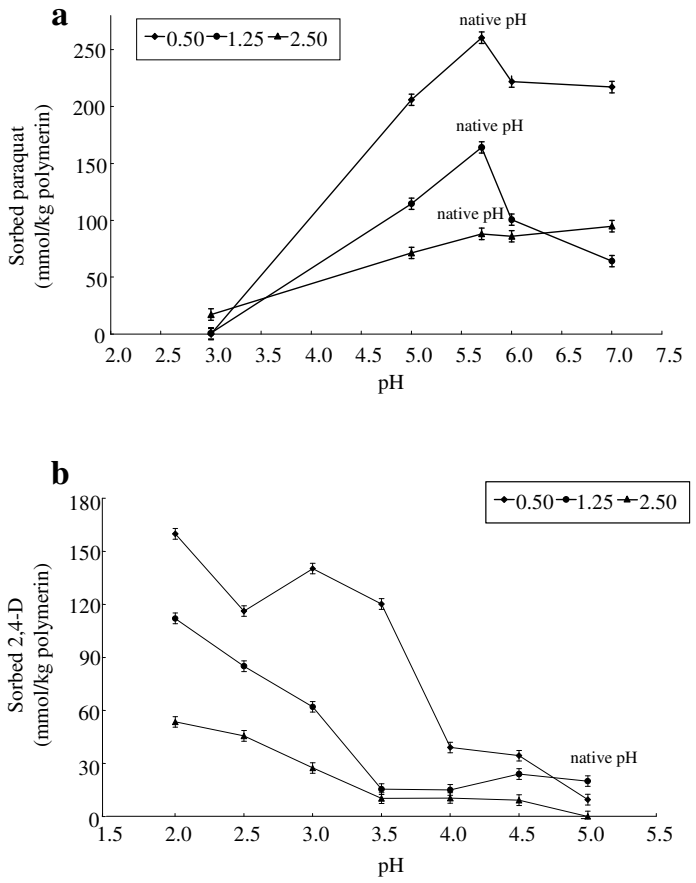


Figure 2. Effect of pH on the sorption of paraquat (a) or 2,4-D (b) by polymerin at solid/liquid ratios of 0.5, 1.25 and 2.5.

Paraquat exists as a double charged species in the pH range that corresponds to the native pH of polymerin, so it could be fixed by ionic bonding. Polymerin could be considered as a weakly acid polyelectrolyte due to its carboxyl and phenol groups.

Polymerin, characterized by a pKa of 4.5 (Capasso et al., 2002) and a pzc of 2.2, tends to exhibit a predominantly negatively charged surface in the pH range 5.0–6.0, so the delocalized positive charge associated with the paraquat is attracted to the sorbent, whereas, no binding was observed at pH 3.0 (Figure 2a). Previous studies (Narine and Guy, 1982) showed similar results for a humic acid/paraquat system, suggesting that the mechanism of interaction was mainly by cationic exchange. These data were in agreement with the chemistry of weakly acidic sites; at a pKa of 5.0, which represents an average value of the acidic functional groups of humic acids (Narine and Guy, 1982); hence it is to be expected that extensive protonation would occur at pH 3.0, with a large reduction in binding of paraquat at this pH value. Also, according to the pzc of polymerin, its surface at pH 3.0 exhibits an increasing number of negatively charged sites that are available due to the loss of H^+ from the surface. As regards 2,4-D [pKa = 2.73, Tomlin (1997)], the ratios of unionized and anionic herbicide are 45:55% and 65:35% at pH 3.0 and 2.0, respectively. The carboxyl groups on polymerin are predominantly in un-dissociated form at this latter pH value which is very close to the value (2.2) of the pzc. So, the stronger sorption of 2,4-D at a pH range 2.0–3.0 (Figure 2b) could involve the formation of H-bonding between the molecular form of the herbicide and the unionized carboxyl groups of polymerin. A significant reduction of sorption of 2,4-D was detected at pH 3.0 (Figure 2b) because the pesticide was in anionic form and the polymerin surface was largely negatively charged causing an increasing repulsion of the pesticide at higher pH.

The effect of time on sorption of paraquat and 2,4-D at pH 5.7 and 3.0 is shown in Figures. 3a and b, respectively.

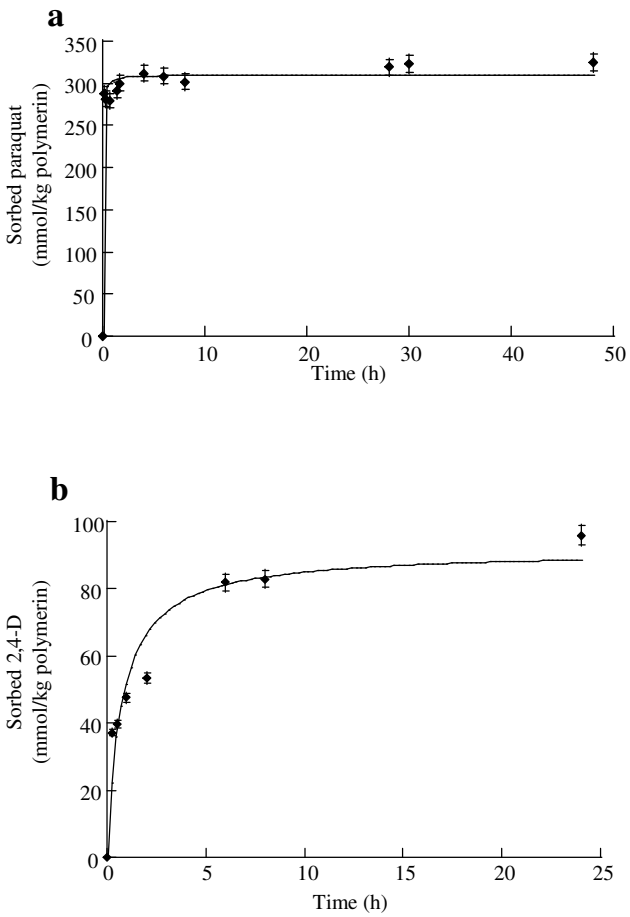


Figure 3. Effect of time on the sorption of paraquat (a) or 2,4-D (b) by polymerin at solid/liquid ratio of 0.5.

In particular for paraquat, the results obtained for all solid/liquid ratios investigated, showed that the process was very rapid, reaching the sorption equilibrium within 1–2h (Figure 3a); no appreciable changes in the sorbed

amount were observed after 4h. The rate constants determined from the slopes of the plots of $\log(q_e\text{-}q)$ versus t varied from 0.218 to 0.147 min^{-1} for the solid/liquid ratios of 0.5 and 2.5, respectively. These results indicated that a first-order mechanism was followed in this process with a higher rate constant at 0.5 ratio. An incubation period of 4h was then adopted to ensure that equilibrium was reached. As regards 2,4-D (Figure 3b), the data showed an increase in sorption up to 24h but this trend was not constant after this time (data not shown). The equilibrium between the two forms of 2,4-D could have a key role in determining this behavior. As the un-dissociated form of 2,4-D binds to the sorbent and is removed from solution, the equilibrium re-establishes by generating further un-dissociated 2,4-D. This dynamic equilibrium could explain the obtained kinetic results.

The sorption isotherms of paraquat (Figure 4a) and 2,4-D (Figure 4b) on polymerin fitted the Langmuir equation. Sorption of both herbicides on polymerin increased slowly with increasing pesticide equilibrium concentration, according to a L-form. Furthermore, the amount of sorbed pesticide was always lower at solid/liquid ratios of 1.25 and 2.5 than at a ratio of 0.5. This behavior was confirmed by analyzing the values of the Langmuir constants, S_m and K, and the distribution coefficients K_d (Table 2). The data also fitted the Freundlich equation, as supported by the high values of R^2 (Table 2). The values of Langmuir and Freundlich constants, K and K_f, and the N values indicated that polymerin sorbed paraquat and 2,4-D with both the greatest binding energy and sorptive intensity at a solid/liquid ratio of 0.5 (Table 2).

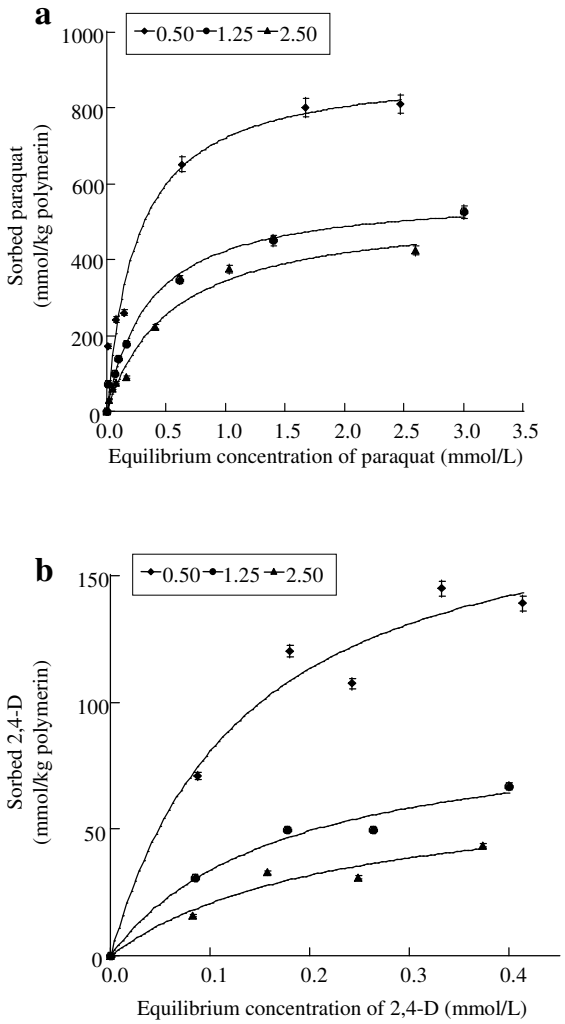


Figure 4. Sorption isotherms of paraquat (a) or 2,4-D (b) by polymerin at solid/liquid ratios of 0.5, 1.25 and 2.5.

Thus, sorption of the pesticides significantly increased with decreasing amount of polymerin. This was attributed to greater accessibility of the sorption

sites, probably associated with a conformational change in polymerin as the solid/liquid ratio changed from 2.5 to 0.5.

TABLE 2. Langmuir and Freundlich Parameters for the Sorption of Paraquat and 2,4-D on Polymerin

	Langmuir parameters			***Freundlich parameters***			K_d [b]
	S_m	K	R^{2a}	K_f	N	R^{2a}	
Paraquat							
Solid/liquid ratio							
0.50	906.95	3.89	0.98	631.10	3.08	0.99	328
1.25	580.82	2.31	0.98	355.55	2.31	0.98	175
2.50	531.80	1.83	0.99	305.42	1.78	0.98	164
2,4-D							
Solid/liquid ratio							
0.50	183.15	8.47	0.98	214.78	2.41	0.98	336
1.25	92.59	5.71	0.98	102.87	2.08	0.98	168
2.50	66.63	4.50	0.98	79.07	1.65	0.99	117

[a]Distribution coefficient.

[b]Correlation coefficient.

3.3.3 Sorption Mechanism

The sorbing capacity of polymerin for the selected herbicides was significant in comparison to other sorbents such as humic substances. In particular, the maximum sorbing capacity of paraquat in our work was of 800 mmol·kg^{-1}, whereas only 500 mmol·kg^{-1} was detected for a similar herbicide (diquat) on a humic acid (Narine and Guy, 1982). In addition, the amount of 2,4-D sorbed on different humic substances (Gianfreda et al., 1996; Celis et al., 1999) was always lower (≈ 37 mmol·kg^{-1}) if compared to that sorbed on polymerin (150 mmol·kg^{-1}). This property supports the possible use of polymerin for remediation, thus the sorption mechanism was investigated.

TABLE 3. Metals Released from Polymerin and Polymerin Loaded with Paraquat or 2,4-D

	Ca	Mg	K	Total
	mmol·kg^{-1} (meq·kg^{-1})			
Polymerin (native pH = 5.7)	45.5 (91.0)	10.5 (21.0)	77.0 (77.0)	133.0 (189.0)
Polymerin + paraquat*	90.0 (180.0)	118.5 (237.0)	322.0 (322.0)	530.5 (738.0)
Metals released after paraquat sorption	**44.5 (89.0)**	**108.0 (216.0)**	**245.0 (245.0)**	**397.5 (549.0)**

	Ca	Mg	K	Total
	mmol·kg (meq·kg^{-1})			
Polymerin (pH = 3.0)	484.5 (969.0)	201.0 (402.0)	387.0 (387.0)	1072.5 (1758.0)
Polymerin + 2,4-D**	805.0 (1610.0)	279.0 (558.0)	398.0 (398.0)	1482.0 (2566.0)
Metals released after 2,4-D sorption	**320.5 (641.0)**	**78.0 (156.0)**	**11.0 (11.0)**	**409.5 (808.0)**

*Sorption experiments were carried out at native pH (5.7) by adding 800 mmol·kg^{-1} (1600 meq·kg^{-1}) of paraquat.

**Sorption experiments were carried out at pH 3.0 by adding 800 mmol·kg^{-1} (800 meq·kg^{-1}) of 2,4-D.

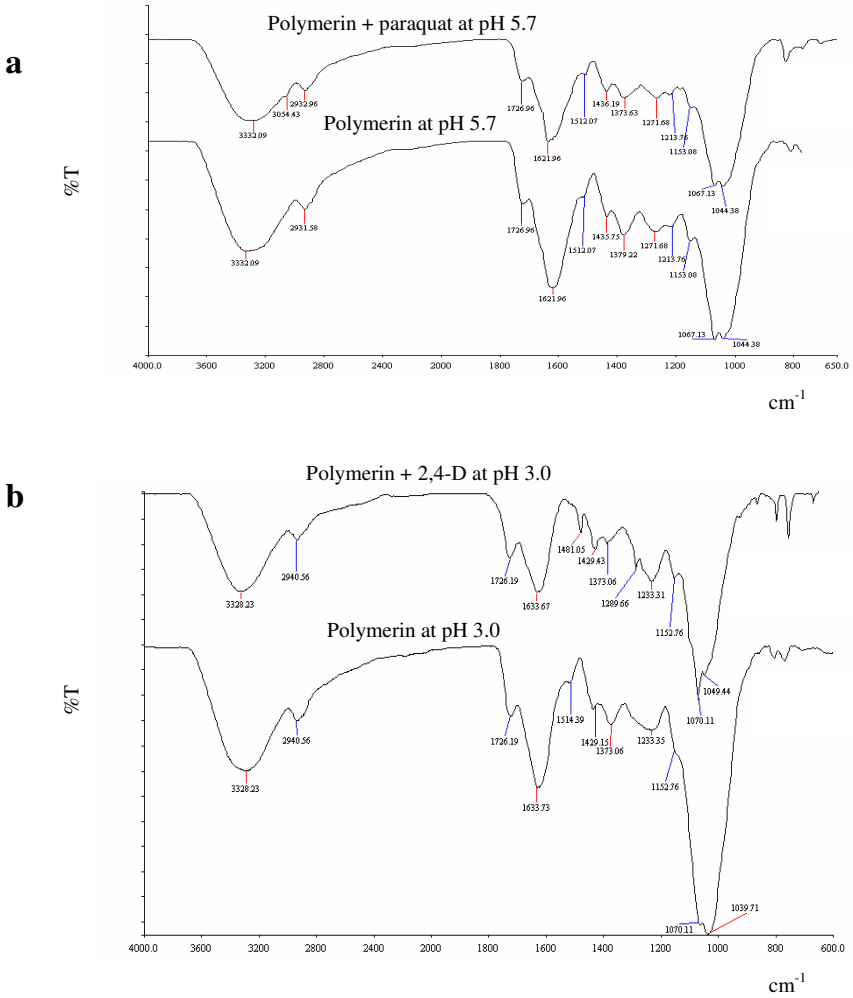


Figure 5. DRIFT spectra of polymerin and polymerin loaded with paraquat (a); DRIFT spectra of polymerin and polymerin loaded with 2,4-D (b).

Table 3 shows data on the sorption of paraquat and 2,4-D on polymerin obtained by adding 800 mmol·kg^{-1} of each herbicide to the sorbent (corresponding to 1600 and 800 meq·kg^{-1}, respectively). The table reports the amount of Ca, Mg and K released from polymerin as a result of sorption of the

herbicides. By adding 800 mmol·kg^{-1} (1600 meq·kg^{-1}) of paraquat to polymerin, 246 mmol·kg^{-1} (492 meq·kg^{-1}) were sorbed and 549 meq·kg^{-1} of Ca, Mg and K were released. This indicates that the sorption mechanism of paraquat on polymerin was consistent with ion exchange of the herbicide with Ca, Mg and K (Table 3). These results are confirmed by previous studies by Narine and Guy (1982) and Spark and Swift (2002) which demonstrated the same sorption mechanism on humic acids and organic matter.

DRIFT spectra recorded at pH 5.7 and 3.0, for paraquat and 2,4-D, respectively, according to the optimum pH sorption of each herbicide, are reported in Figures. 5a and b. The DRIFT analysis (Figure 5a) of polymerin loaded with paraquat shows the appearance of one shoulder at 3054 cm^{-1} and an increase of the peak at 824 cm^{-1} as compared to the corresponding peak at 812 cm^{-1} in the DRIFT spectrum of original polymerin: the new bands may be assigned to the quaternary ammonium and pyridine rings of the herbicide, respectively.

Results reported in Table 3 for 2,4-D showed that by adding 800 mmol·kg^{-1} of herbicide to the sorbent, 145 mmol·kg^{-1} (145 meq·kg^{-1}) were sorbed and 808 meq·kg^{-1} of Ca, Mg and K were released. The native pH of polymerin (5.7) decreased to pH 5.0 by adding 2,4-D to the sorbent and, in solution, the total amount of metals released after sorption (808 meq·kg^{-1}) was almost equal to the quantity of 2,4-D added (800 meq·kg^{-1}). To explain these data, we hypothesize that 2,4-D, being a stronger acid (pKa = 2.73) than polymerin (pKa = 4.5), released protons which bound to -COO^-K^+, -$(COO^-)_2Ca^{++}$ and -$(COO^-)_2Mg^{++}$ groups of the sorbent, liberating these cations into solution. Under these experimental conditions (pH = 5.0), 2,4-D was present only in its anionic form. When the pH of the suspension changed from 5.0 to 3.0 (optimum pH for sorption experiments), the equilibrium between the anionic and acidic form of 2,4-D was restored, and in this latter form the herbicide bound to polymerin by

hydrogen bonding. This kind of interaction was also observed by Piccolo (1994) and Gianfreda et al. (1996) in sorption studies of this herbicide on humic acids. The DRIFT spectrum (Figure 5b) of polymerin loaded with 2,4-D shows the appearance of two new bands at 1481 and 1289 cm^{-1}, assignable to C=C and C–O–C of aromatic ethers, respectively. In addition, the bands at 1633 and 1070 cm^{-1}, mainly arising from the carboxylic groups and alcoholic groups of the polysaccharide component, decreased significantly. These results support the conclusion that the acidic form of 2,4-D bound on polymerin by hydrogen bonding with the carboxylic and alcoholic groups of the sorbent.

3.3.4 Cyclic Removal of Pesticides by Polymerin from Simulated Wastewaters

Experiments on cyclic sorption investigated the potential to totally remove the pesticides from simulated wastewaters.

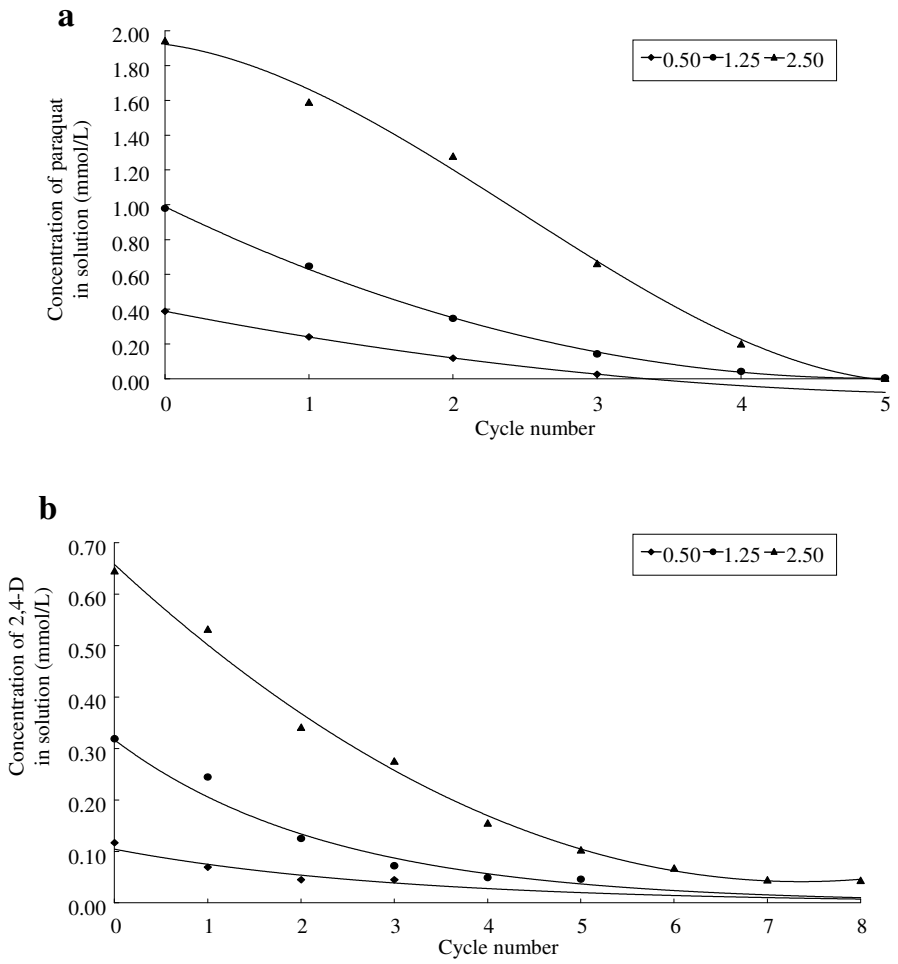


Figure 6. Removal of paraquat (a) or 2,4-D (b) from solutions at solid/liquid of 0.5, 1.25 and 2.5, by cyclic sorption on polymerin (the bio-sorbent was renewed in each cycle).

Figure 6a shows that total removal of paraquat from solution was achieved after three, five and four cycles at 0.5, 1.25 and 2.5 solid/liquid ratio, respectively. In contrast Figure 6b shows a significant but incomplete removal of 2,4-D. Furthermore, the quantity of removed 2,4-D remained constant after two cycles at a ratio of 0.5. No further removal was observed for ratios of 1.25 and 2.5 after four and seven cycles, respectively. The maximum decontamination of simulated wastewaters polluted by 2,4-D at ratios of 0.5, 1.25 and 2.5 was 44%, 66% and 79%, respectively (Figure 6b). These data indicate that a threshold concentration value (0.04 $mmol \cdot L^{-1}$) existed for 2,4-D at which no sorption occurred, and this value was independent on the amount of sorbent and the number of cycles. This fact suggests that at the concentration of 0.04 $mmol \cdot L^{-1}$ the acid–base equilibrium of 2,4-D was strongly in competition with the sorption equilibrium between the un-dissociated form of 2,4-D and polymerin.

3.4 Conclusions

The capacity of polymerin at native pH to totally remove paraquat from contaminated wastewaters identifies this bio-sorbent as a suitable bio-filter for the decontamination of point source pollution. The sorbent is an anionic polyelectrolyte and should thus be versatile for the sorption of other cationic pesticides, such as s-triazines, that are in cationic form at pH 5.7. In addition, the sorption causes the release of K, Mg and Ca from the sorbent which are macro-elements essential in plant and animal nutrition. Thus, this kind of water can be potentially recycled for irrigation in the same rural zones where point source pollution occurs. Polymerin appears to be less suitable for the decontamination of waters containing 2,4-D as it leaves residual herbicide even after many sorption cycles. Thus, the combination with a cationic sorbent (e.g. ferrihydrite) or, alternatively, the immobilization of the bio-sorbent on the cationic sorbent, may be necessary for the total removal of the pesticide from polluted waters. However, evaluating the pKa (4.5) and the pzc (2.2) of polymerin, this latter may be used for acid pesticides with a pKa of around 5.0. In fact, at a pH 3.0 this kind of pesticide should be totally in un-dissociated form and polymerin near to the pzc (2.2), avoiding a significant negative repulsion between the polymerin surface and the pesticide. Even in this case a release of K, Mg and Ca from polymerin should be observed. Acidic pesticides potentially suitable to extend the application of the model sorption of 2,4-D on polymerin, could be bentazone and aryloxyphenoxypropionates. The exploitation of this bio-sorbent recovered from OMW should also contribute to resolve the disposal problems of these wastewaters, which are produced in high quantity in all of the countries where olive oil is produced.

CHAPTER 4

Sorption of New Generation and Currently Used Herbicides on Polymerin: The Study of Cyhalofop Acid[†]

Abstract

A study on the sorption of the acid form of the herbicide Cyhalofop-butyl on polymerin is here reported. Cyhalofop-butyl is a chemical recently introduced from Dow AgroSciences for the control of *Echinochloa spp* in the rice culture. The ester presents a low water solubility, but it hydrolyzes, rapidly, to acid, much more soluble and responsible of the effective herbicide action. Cyhalofop-acid showed to be sorbed on polymerin to a maximum amount of 145 mmol kg^{-1} at pH around 4.5 at solid/liquid ratio of 0.5, very likely by H-bonding. Cyhalofop-acid proved to be removed from simulated waste waters contaminated with the herbicide by eight cycles of sorption on polymerin renewed at each cycle, suggesting this bio-sorbent a possible bio-filter to be used for the decontamination of point source pollution.

[†] A version of this chapter has been published.
Iorio, M., Sannino, F., De Martino, A., Pinna, M.V., Capasso, R. (**2007**). Removal of cyhalofop acid from aqueous solutions by sorption on polymerin. In: *Environmental fate and ecological effects of pesticides* (AA:M. Del Re, E. Capri, G. Fragoulis and M. Trevisan (Eds) La Goliardica Pavese (Italy), pp. 607-611 (ISBN 978-88-7830-473-4).

4.1 Introduction

Pesticides play an important role in the success of modern farming and food production. However, the use of pesticides has been a public concern for decades, owing to their potential risk to both the human health and the environment. Surface waters and groundwaters have been shown to be contaminated with a wide range of pesticides (De Wilde et al., 2007).

Research over recent years has been focused on the non-point sources of pesticide contamination resulting from application to agricultural land. However, contamination arising from point sources such as areas on farms where pesticides are handled and filled into sprayers, and where sprayers are cleaned, non-approved use, poor practice, illegal operations and accidental releases from the farmyard, is increasingly recognized as contributing to water contamination (Carter et al., 1999; Yoder et al., 2001; Kruger et al., 2001).

Several workers have indicated that the point sources can contribute between 18 and 84% of the pesticide load measured in individual catchments (Higginbotham et al., 1999; Muller et al., 2002). Better training of sprayer operators and good machinery maintenance can reduce the number of spills. Furthermore, releases to the farmyard due to spray tank washings must to be minimized (Rose et al., 2001). The application of treatment systems at the farm level could allow an improved management of the aquatic ecosystem and a reduction of water treatment costs.

The possibility of using vegetable biomass wastes as a biofilter for the remediation of waste waters generated at point sources was described by Pussemier et al. (1998). The olive oil production is a significant agricultural activity with a great economic importance particularly in Mediterranean countries. However, it generates high amounts of waste waters deriving from the olive mill process (olive mill wastewater or OMW). This waste causes disposal

problems because of its potential polluting properties, which are documented by high chemical oxygen demand (COD) and biological oxygen demand (BOD) (Arienzo and Capasso, 2000). Nevertheless, OMW may also be regarded as an inexpensive source of vegetable biomass to be recovered for use in environmental biotechnology processes (Capasso et al., 2004) with the potential advantage to eliminate the environmental impact deriving from its discharge.

The use of vegetable biomass with high sorption capacity for remediation of waters contaminated with pesticides or metals has been widely described in the literature over the last ten years (Schneegurt et al., 2001; Mameli et al., 2004). In particular, the utilization of the polymeric organic fraction of OMW, named polymerin (Capasso et al., 2002), for remediation of contaminated waters with pesticides could represent a convenient method of bioremediation. This material derives from a cost-free agricultural waste and possesses humic acid-like characteristics. Previously, we demonstrated that polymerin exhibits strong sorption properties either for cationic and anionic heavy metals (Capasso et al., 2004; Sannino et al., 2006) or ionic and ionizable pesticides, such as paraquat and 2,4-D, used as models (Sannino et al., 2007).

Since the studies carried on sorption of models have shown positive results, next step was to investigate the potential employment of polymerin as bio-filter for the removal of new generation and currently used pesticides. Thus, the aim of this work was to study the sorption of cyhalofop acid (CyH) by polymerin. Cyhalofop-butyl (CyB), 2-[4-(4-cyano-2-fluoro-phenoxy)phenoxy]propanoic acid, butyl ester (R), recently introduced from Dow AgroSciences, is an aryloxyphenoxypropionate herbicide for the post-emergence control of grasses in rice, mainly against almost all *Echinochloa* species. The ester presents a low water solubility, but it hydrolyzes rapidly into its corresponding derivative 2-[4-(4-cyano-2-fluorophenoxy) phenoxy] propionic acid (CyH), which is much more soluble and presents the effective herbicide action.

4.2 Experimental Section

4.2.1 Preparation and Characterization of Polymerin

Polymerin was prepared and characterized according to the procedure previously reported by Capasso et al. (2002). An aliquot of OMW (year 2004) from a pressure processing system located in Monteroduni (Italy) was transformed into the complex polymeric mixture.

4.2.2 Chemicals

CyB was purchased from Sigma-Aldrich Chemical Company (Poole, Dorset, UK; 99.0% purity). Its corresponding derivative, CyH (Figure 1), was prepared by basic hydrolysis of CyB. In particular, an aqueous solution of NaOH was added to a suspension of CyB (1 g) in 100 mL of CH_3CN/H_2O 50/50, under stirring at room temperature, up to pH 9.0

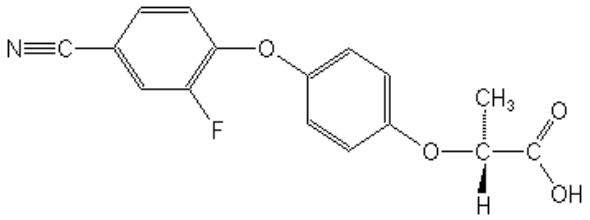


Figure 1. Structure formula of Cyhalofop acid.

. The pH of the mixture was checked time course and brought to value of 9.0 by NaOH addition until the suspension turned into solution. CH_3CN was

evaporated and diluted HCl was added to solution: a white precipitate was obtained which was filtered and repeatedly washed with H_2O to give pure CyH. All the solvents were of HPLC grade (Carlo Erba, Milan, Italy) and were used without further purification. All other chemicals were obtained from Sigma-Aldrich unless otherwise specified.

4.2.3 Sorption Experiments

4.2.3.1 *Sorption Methodology*

Stock solution of pesticide was prepared by dissolving 125 mg of CyH in 500 mL of KCl 0.03 M and alkalizing the solution at pH 10.0 with KOH (final concentration 0.830 mmol·L^{-1}). This solution was then kept refrigerated. The sorption experiments were conducted at solid/liquid ratio of 0.5 obtained by adding 10 mg of polymerin, previously dried at 100 ±1°C for 1 h, to a final volume of 20 mL at 20 ±1 °C. The pH was controlled by addition of 0.01 mmol·L^{-1} HCl or KOH to the suspension. After the equilibration time, the samples were centrifuged at 5500 *rpm* for 30 min using Centrifugal Filter Devices equipped with Amicon Ultra membrane with cut-off of 5000 Da. The amount of herbicide sorbed on polymerin was calculated as the difference between the initial concentration of pesticide added and that present in the equilibrium solution.

4.2.3.2 *Analytical Determination of the Herbicide*

CyH was analyzed with an Agilent 1200 Series HPLC equipped with a DAD array and a ChemStation Agilent Software. A Macharey-Nägel Nucleosil 100-5

C18 column (stainless steel 250 x 4 mm) was utilized. An isocratic elution was performed at a flow rate of 1.0 mL·min^{-1} with CH_3CN (50%) and phosphate buffer (50%) (0.04 M, pH 2.95). The wavelength utilized was 250 nm. A sample volume of 20 µL was used for injection.

The quantitative determination of CyH was performed elaborating the corresponding calibration curve in a range of 0.016-0.75 mmol·L^{-1}. Polymerin blanks and CyH controls, treated in the same conditions of the samples, were used.

4.2.3.3 Effect of pH

Experiments were carried out by adding a fixed pesticide concentration of 0.17 mmol·L^{-1}, and varying the pH (from 3.0 to 11.0). The suspensions were shaken for 24 h. The herbicide was separated from the sorbent and analyzed as described below.

4.2.3.4 Sorption Isotherms

Different volumes of a stock solution of the herbicide (0.83 mmol·L^{-1}) were added to polymerin to give an initial concentration ranging from 0.040 to 0.70 mmol·L^{-1} of CyH. The pH of each suspension was kept constant at pH 4.5 for 24 h by addition of 0.10 or 0.01 mol·L^{-1} HCl or KOH.

The sorption data were analyzed according to the Langmuir equation, that can be written as follows:

$$S = S_m K C_e / (1 + K C_e) \qquad (1)$$

where S is the amount of pesticide sorbed on polymerin (mmol·kg^{-1}), K is the Langmuir constant related to the binding energy, S_m is the maximum amount of

pesticide sorbed (mmol·kg^{-1}) and *C*e is the equilibrium concentration of pesticide (mmol·L^{-1}), according to Giles et al. (1974).

4.2.4 Cyclic Sorption on Polymerin

Cyclic experiments for sorption of CyH were carried out at pH 4.5 and each cycle had a duration of 24 h. In the first cycle a predetermined amount of herbicide stock solution was pipetted into the flasks to give 0.664 mmol·L^{-1} of CyH. The samples, with a final volume of 20 mL, were then shaken for 24 hours, centrifuged and the filtrates were analyzed to determine the amount of the sorbed pesticide. For the second cycle, the filtrate was made up to 20 mL before adding fresh polymerin (10 mg). The experiments were repeated until the equilibrium concentrations remained constant.

4.3 Results and Discussion

4.3.1 Characterization of the Sorbent

Polymerin was recovered from OMW and characterized chemically and spectroscopically (DRIFT) according to the procedure described by Capasso et al. (2002). The material was confirmed to be a humic acid–like polyelectrolyte, consisting of carbohydrates, melanin and proteins. It also contains metals such as Ca, Mg, K, Na, and Fe (III), that are naturally bound and chelated through carboxylate anions and other characteristic nucleophilic functional groups present in polymerin.

4.3.2 Sorption Studies

The results obtained as function of pH and reported in Figure 2, showed that the sorbed amount of the herbicide was significant at pH 4.0-4.5. Therefore, the optimum pH fixed for the sorption studies was 4.5.

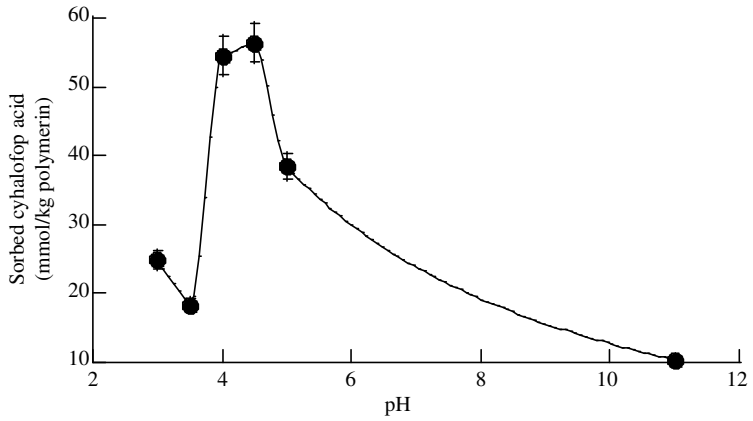


Figure 2. Effect of pH on the sorption of cyhalofop acid on polymerin.

Polymerin could be considered as a weakly acid polyelectrolyte due to its carboxyl and phenol groups. Sorption process may be very probably attributed to the formation of hydrogen-bonding between the alcoholic groups of the polysaccharide component of the sorbent and the nitrile group, the ethereal oxygen and the un-dissociated carboxylic group of the herbicide. At pH 4.5, the effect of H-bonding is highly competitive with respect to the repulsion between the partially ionized carboxylic groups of polymerin (pK_a=4.5) and dissociated form of the CyH (pK_a = 3.8). In contrast, a significant reduction of sorption of CyH was detected at pH > 4.5 because the pesticide and polymerin surface were largely negatively charged, causing an increasing repulsion of the pesticide. In

this case, the repulsion effect should be more competitive as compared with the binding effect of the H-bonding.

The sorption isotherm of the herbicide (Figure 3) on polymerin fitted the Langmuir equation. In fact, the sorbed amount increased slowly with increasing pesticide equilibrium concentration, according to a L-form.

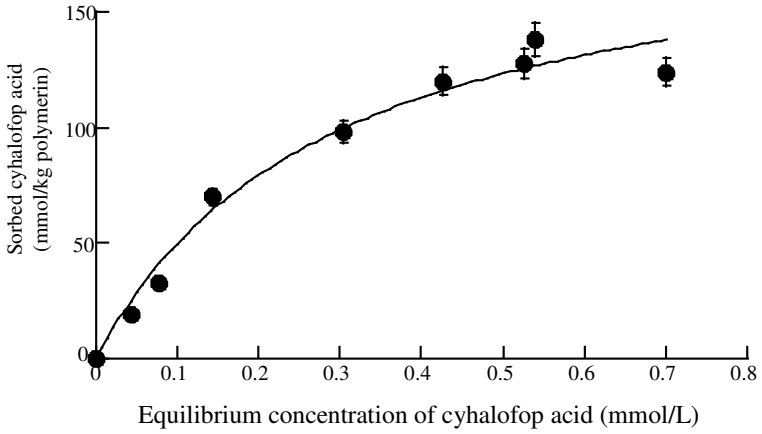


Figure 3. Sorption isotherm of cyhalofop acid on polymerin.

The sorbed quantity of CyH detected on polymerin (145 mmol·kg^{-1}) was to a similar extent compared with that of 2,4-D, an acidic herbicide previously studied (Sannino et al., 2007), suggesting for both the herbicides a similar behavior.

4.3.3 Cyclic Removal of Cyhalofop Acid by Polymerin from Simulated Wastewaters

Experiments on cyclic sorption investigated the potential to totally remove the herbicide from simulated waste waters. Figure 4 shows that CyH was totally removed from the contaminated solution after 8 cycles, according to the sorption mechanism above proposed, as the effect of H-bonding is strongly in competition with the repulsion of the ionized carboxylic groups of the sorbent and the dissociated form of the herbicide.

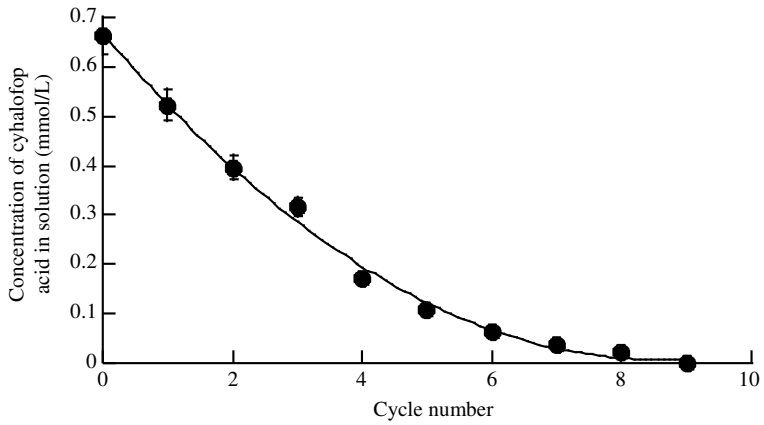


Figure 4. Removal of cyhalofop acid from solutions by cyclic sorption on polymerin (the bio-sorbent was renewed in each cycle).

4.4 Conclusions

With this study we report for the first time CyH to be able to be sorbed on polymerin. Eight cycles of sorption of CyH on polymerin, renewed at each cycle, removed totally the herbicide from simulated waste waters, suggesting polymerin to be a possible bio-filter for the decontamination of point sources. In addition, the exploitation of this bio-sorbent recovered from olive oil mill waste waters, could contribute to resolve the disposal problems of these wastes, which are produced in high quantity in all of the countries where olive oil is produced.

CHAPTER 5

Sorption of Phenanthrene by Polymerin Associated with Micro- and Nanoparticles of Aluminum Oxides[‡]

Abstract

Intent of this study was to explore the potential application of polymerin, the polymeric organic fraction from olive oil wastewaters, in technologies aimed at remediating hydrophobic organic compounds (HOCs) point-source pollution. Sorption of phenanthrene by polymerin was investigated. Moreover, the effect of the addition of micro and nanoscale aluminum oxides (Al2O3) was studied, as well as sorption of polymerin on the oxides. Phenanthrene sorption capacity by polymerin was notably higher than both Al2O3. Polymerin sorption on nanoparticles was nearby 100 times sorption on microparticles. In a three-phase system, when using the microparticles, higher Phen sorption was found by adding into water polymerin, oxides and Phen simultaneously. In contrast, when using the nanoparticles, a considerable enhancement of Phen sorption was shown by adding Phen to a pre-formed and dried polymerin-oxide complex. These findings support the application of polymerin, especially associated with aluminum oxide nanoparticles, in remediation of water contaminated with HOCs.

[‡] A version of this chapter has been submitted for publication.
Iorio, M., Pan, B., Capasso, R., Xing, B. Sorption of phenanthrene by polymerin and its complex with aluminum oxide nanoparticles. *Environmental Pollution*. December, **2007**.

5.1 Introduction

The necessity to develop new technologies aimed at mitigating the risk deriving from soil and water pollution is constantly increasing. Accidental releases, illegal operations or not-appropriate disposal of wastes from industries and farms are the main causes of point sources of soil and water pollution by several kinds of compounds, from pesticides to hydrophobic organic chemicals (HOCs). One tool for the reduction of point source contamination is the use of *biobeds*. These systems can be represented by a column with layers of straws, peat moss and top soil, being able to adsorb and degrade pollutants from water, and they were introduced by Torstensson (1997) and then improved by numerous researchers (Spliid et al., 2006; Coppola et al., 2007; Fait et al., 2007). At this aim, it is important to find new and cheap sorbents to better develop this technique. Several studies propose the use of natural organic materials or wastes as bio-sorbent (Rodriguez-Cruz et al., 2007; Wang et al., 2006; Wang et al., 2007) due to the high sorption capacity of organic matter (Kang and Xing, 2005; Gunasekara and Xing, 2003).

Olive oil mill wastewater (OMW) is a vegetable biomass waste produced in large amounts, especially in Mediterranean countries, which are the main producers of olive oil worldwide. Its disposal represents a significant problem for these countries because OMW is a potential pollutant due to its high COD and BOD values (Arienzo and Capasso, 2000), and also because it is produced in large quantity in a very short period of a year. However, OMW may also be regarded as a source of useful products. In this light, we recovered the mineral-organic fraction, named polymerin, showing polyelectrolyte and humic acid-like properties (Capasso et al., 2002). High sorbing capacity of metals and ionic and ionizable pesticides by polymerin was observed (Sannino et al., 2006; Capasso et al., 2004; Sannino et al., 2007). Thus, it could represent a cheap sorbent of

potential economic interest and, at the same time, its use could minimize the OMW disposal problem. Some studies considering the sorption of polar compounds on polymerin were carried out, but its interaction with HOCs is unknown. For this study we choose phenanthrene as a representative HOC.

Sorption of heavy metals on the polymerin complexed with ferrihydrite (Sannino et al., 2006; Capasso et al., 2004) was recently studied because of its significant capacity to adsorb ions. The obtained results showed an effective enhancement of the sorption capacity of the complex in comparison with either polymerin or ferrihydrite used individually. In this work we selected different sized-aluminum oxides to interact with polymerin and phenanthrene in order to investigate the sorption capacity variation. This mineral possesses a high surface area with positive charges and due to the hydration layer it is not an effective sorbent for HOCs. By coating the oxides with a negatively charged organic matter, it is possible to change their hydrophilic surface into hydrophobic, which have a great affinity for HOCs. Several studies suggest the application of aluminum oxides (Al_2O_3) coated with anionic surfactants in wastewater treatment techniques (Park and Jaffè, 1993; Valsaraj et al., 1998). Polymerin could represent an advantageous alternative to the use of surfactants. Moreover, since polymerin by itself is soluble in water, it cannot be readily used in biobeds, which need solid materials. Thus, by coating Al_2O_3 with polymerin we may obtain a new solid sorbent for a biobed system. Therefore, the main objective of this work was to investigate the behavior of phenanthrene in water in the presence of polymerin and in a polymerin-oxide-water three-phase system. Moreover, possible application of these sorption systems as a bio-filter for remediation of pollution point sources is discussed.

5.2 Experimental Section

5.2.1 Sorbates and Sorbents

An aliquot of OMW (year 2004) from a pressure processing system located in Monteroduni (Italy) was processed in order to obtain the complex polymeric mixture. Polymerin was characterized according to the procedure previously reported by Capasso et al. (2002a). Microscale aluminum oxide was purchased from J. T. Baker Chemical Co. (New Jersey) and nanoscale aluminum oxide from Hongchen Material Sci. & Tech. Co., Zhejiang, China. Ring-UL-^{14}C labeled and unlabelled phenanthrene (Phen) were purchased from Sigma-Aldrich Chemical Co.

TABLE 1. Properties and Chemical Composition of Sorbents and Sorbate.

Mineral sorbents:	Al_2O_3 microparticles	Al_2O_3 nanoparticles
Surface area (m^2g^{-1})	32	228
Point of zero charge, PZC	7.30	8.50
Mean particle size (μm)	110	0.06*
Organic sorbent:	**polymerin**	
Chemical composition (%, w:w)		
polysaccharide	43.07	
protein	22.40	
melanin	29.76	
metals	4.77	
carbon	41.49	
Range of molecular weight (Da)	first peak: 3500-10000 second peak: 45000	
Organic pollutant:	**phenanthrene**	
Molecular weight	178	
Aqueous solubility	1.29 mg L^{-1}	
Octanol-water partition constant, log Kow	4.57	

*provided by the supplier.

They were used without any further treatment. Specific surface area of the particles was determined by H_2O sorption at 20% relative humidity using the

method by Quirk (1955). Point of zero charge (PZC) of both aluminum oxides was measured using a Zetasizer (Malvern Instruments). Microscale particle size was analyzed by static light scattering (Coulter LS230). Chemical composition and physical properties of sorbents and sorbates are listed in Table 1.

5.2.2 Sorption Experiments

5.2.2.1 Adsorption of Phenanthrene on Aluminum Oxides

Adsorption isotherms on both micro and nanoparticles were obtained by a batch equilibration technique. Solid-to-solution ratios were adjusted to reach 30-80% solute uptake by sorbents. Due to its low water solubility, Phen stock solution was prepared in methanol at high concentrations. ^{14}C labeled and unlabeled Phen were mixed to achieve a concentration gradient from 10 to 1000 $\mu g \cdot L^{-1}$ in water. These solutions were added to 15 mL screw cap vials containing 500 mg or 20 mg of Al_2O_3 microparticles or nanoparticles, respectively, until a minimum headspace was achieved. Methanol concentrations were kept below 0.1% of the total solution volume. Vials were immediately sealed with aluminum foil-lined Teflon screw caps and then mixed on a shaker for 3 days at 23 ± 1 °C. After mixing, the vials were centrifuged at 3000 rpm for 30 min, and 1 mL of supernatant was removed and added to 7 mL Scintiverse cocktail (Fisher Scientific Co., Pittsburgh) for liquid scintillation counting (LSC) (Beckman LS 6500, CA). Adsorbed Phen on Al_2O_3 was determined by mass balance, because of the negligible mass loss of solute.

5.2.2.2 Adsorption of Polymerin on Aluminum Oxides

Adsorption of polymerin on Al_2O_3 was carried out by diluting a 5000 $mg \cdot L^{-1}$ (2000 $mgOC \cdot L^{-1}$) stock solution of polymerin, in a range from 10 to 400 $mgOC \cdot L^{-1}$, in 15 mL screw cap vials containing 500 or 20 mg of micro and nanoparticles, respectively. After 3 days of equilibration, vials were centrifuged and supernatants were analyzed by an Agilent (Palo Alto, CA) 8453 UV-Visible spectrometer set at 254, 280, 465 and 665 nm. Uptake of polymerin by particles was obtained by mass balance.

5.2.2.3 Sorption of Phenanthrene by Polymerin

Sorption isotherms of Phen by polymerin were obtained by a batch equilibration technique using 3500 molecular weight cut-off (MWCO) regenerated cellulose dialysis tubing (Spectra/Por 3, Spectrum Laboratories, CA), as described by Pan et al. (2007). During polymerin preparation, the matrix was dialyzed using 3500 MWCO dialysis membrane. This procedure assured to avoid sorbent loss during the sorption experiments. 40 $mgOC \cdot L^{-1}$ solution of polymerin was added inside a 10 cm length dialysis bag and an increasing concentration of Phen in the same range used for adsorption on the Al_2O_3 particles was added outside the bag in a 15 mL screw cap vial. The equilibration procedure was the same as described above. After 3 days 1 mL solution from both inside and outside the bag was collected and analyzed by LSC. Uptake of solute by polymerin was calculated by the difference between inside and outside amounts of Phen (Pan et al., 2007).

5.2.2.4 Three-Phase Sorption

Three phase sorption experiments were carried out in two different ways: i) by keeping constant Phen concentration and varying polymerin concentration and ii) by using a fixed amount of polymerin and different concentrations of Phen. In the first case, increasing concentrations of polymerin, ranging from 0 to 600 mgOC·L^{-1}, were added to 500 or 20 mg of micro or nanoscale Al_2O_3 particles, respectively, together with 1000 µg·L^{-1} of Phen using a batch equilibration method. In the second case, samples were divided into three different batches prepared as schematically reported in Figure 1. Three-phase samples (T-samples) were prepared by adding particles, polymerin and Phen simultaneously; after three days samples were centrifuged and the supernatant was analyzed by LSC. Complex samples (C-samples) were performed by shaking particles and polymerin for three days in order to create the complex. Then, samples were centrifuged and 70% of supernatant was collected and used to prepare Phen stock solution to add to the samples. After three days the samples were centrifuged and the supernatant was analyzed by LSC. Dried samples (D-samples) were prepared following the same protocol used for C-samples, but, before adding Phen solution, the complex (30% remaining in the vials) were freeze-dried (Figure 1). Phen stock solution was prepared in the polymerin solution remaining after adsorption on the particles in order to keep a constant concentration of polymerin in the system in all of the different batches. Moreover, polymerin solution remaining after adsorption on both micro and nanoparticles was collected and sorption isotherms of Phen were obtained using the dialysis bags method as described above. All the experiments were conducted in duplicate

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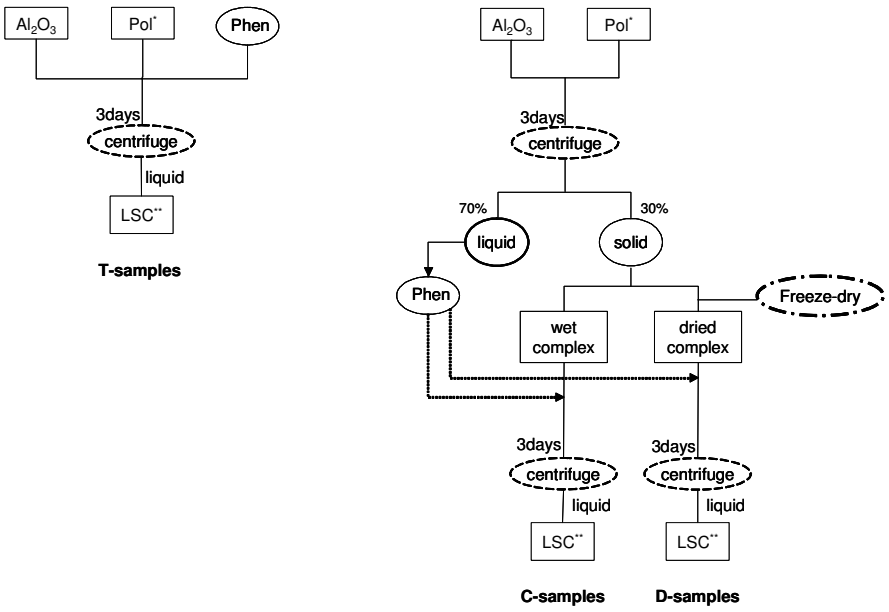

Figure 1. Flow chart of the experimental design used to study phenanthrene behavior in a three-phase system. *Pol is the abbreviation of polymerin. **LSC is the abbreviation of Liquid Scintillation Counter. *Liquid* represents polymerin solution remaining after adsorption on particles. *Solid* represents the polymerin-particles complex.

5.2.2.5 Sorption Isotherms Models

According to the type of interaction occurring between sorbent and sorbate, we used different models to fit the data, such as the logarithmic form of Freundlich equation, Langmuir equation and a linear model.

The linearized form of the Freundlich equation is:

$$\log S = \log K_f + N \log C_e \qquad (1)$$

where *S* is the solid-phase concentration of Phen ($\mu g \cdot kg^{-1}$) and *C*e represents the aqueous-phase concentration of Phen ($\mu g \cdot L^{-1}$). K_f [$(\mu g \cdot kg^{-1})/(\mu g \cdot L^{-1})^N$] and *N* (dimensionless) are the Freundlich sorption parameters.

Langmuir equation was employed to fit polymerin sorption on mineral particles:

$$S = S_m K C_e / (1 + K C_e) \quad (2)$$

where *S* is the amount of polymerin sorbed on particles ($mgOC \cdot kg^{-1}$), *K* is the Langmuir constant related to the binding energy, S_m is the maximum amount of polymerin sorbed ($mgOC \cdot kg^{-1}$) and *C*e is the equilibrium concentration of polymerin ($mgOC \cdot L^{-1}$)

The single point sorption coefficient was used widely for comparisons in the literature due to isotherm nonlinearity (Wang et al., 2006). Therefore, the single point K_d was calculated based on Freundlich equation at a given concentration (*C*i):

$$K_d = S / C_i = K_f \times C_i^{n-1} \quad (3)$$

5.2.2.6 *Mathematical Corrections Used in K_d Calculation for Three-Phase Systems*

In a three-phase system Phen can be adsorbed by three different sorbents: i) particles, ii) polymerin, and iii) polymerin-particle complex. In the solid phase it is possible to find free particles and the complex; liquid phase consists of un-adsorbed polymerin and water. Freundlich parameters for all the three phase isotherms are shown in Table 1. For each batch, K_d* (apparent K_d) was calculated.

$$K_d^* = S / C_e = K_f \times C_e^{n-1} \quad (4)$$

Successively, it was adjusted in the denominator by subtraction of Phen bound on polymerin (C_b) from aqueous Phen, obtaining K_d1.

$$C_{free} = C_e - C_b \quad (5)$$

$$K_d 1 = S / C_{free} = K_f \times C_{free}^{n-1} \quad (6)$$

This correction was necessary to calculate the real amount of Phen distributed between solid particles and water. An additional correction was done in the numerator of K_d1 by subtraction of adsorbed Phen on free particles from the solid phase, obtaining K_d2.

$$S_{complex} = S - S_{particles} = S - K_f \times C_{free}^{n} \quad (7)$$

$$K_d 2 = S_{complex} / C_{free} \quad (8)$$

This subtracted value was representative of the maximum contribution given by particles, even if they were engaged in complex formation, therefore it would be overestimated.

5.2.3 Spectroscopic Analysis

The ratio of absorbance of polymerin at 465 and 665 nm (E_4 to E_6 ratio) was determined from aqueous solution remaining after adsorption on micro and

nanomaterials. We collected and analyzed each supernatant from polymerin-particles sorption experiments. The same solutions were also analyzed by Fourier Transform Infrared Spectroscopy (FT-IR) using a Perkin-Elmer spectrometer (Spectrum One) equipped with a Universal ATR Sampling accessory (Kang and Xing, 2007).

5.3 Results and Discussion

5.3.1 Al_2O_3 Particles Size Considerably Affected Polymerin Sorption

Freundlich and Langmuir parameters for the sorption studies are listed in Table 2. Both Al_2O_3 micro and nanoparticles had a nonlinear sorption of polymerin (Figure 2), as highlighted by the *N* values (0.63 and 0.43) from the logarithmic form of the Freundlich equation (Table 2).

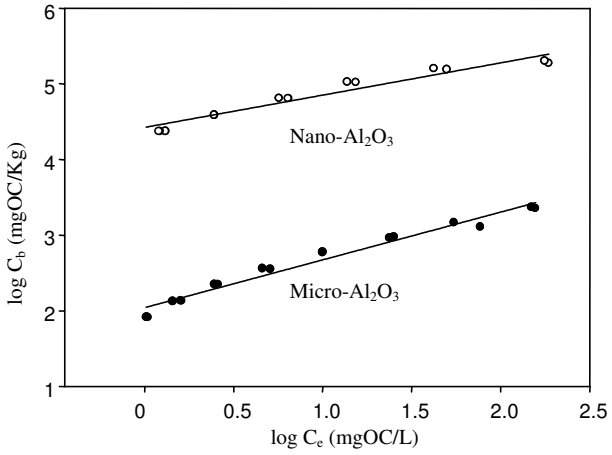

Figure 2. Sorption isotherms of polymerin by micro and nanoparticles of aluminum oxides.

By comparing R^2 from both Freundlich and Langmuir equations, polymerin sorption on the particles follows a Langmuir-type trend ($R^2 \geq 0.99$). The sorption capacity of nanoparticles (S_m) was almost two orders of magnitude higher than that of microparticles (S_m = 203800 and 2970 $mgOC \cdot Kg^{-1}$, respectively). Moreover, nanoparticle Langmuir adsorption constant (*K*) was higher than microparticle's constant. Since elemental composition was identical,

the first reasonable explanation for such a large difference was initially related to the surface area. In fact, nanoparticle surface area was considerably higher than microparticle (228 and 32 m^2g^{-1}, respectively). But, even if surface area significantly influenced the sorption, it was not enough to explain the two orders of magnitude enhancement. Nano-to-micro surface area ratio was only seven (228/32). Another investigated parameter was zeta potential. Microparticle's PZC was lower than nanoparticles (7.30 and 8.50, respectively), hence micro-Al_2O_3 might have a lower charge density. This factor also may influence polymerin sorption due to the electrostatic attraction. However, sorption experiments were conducted at pH 6.0-6.4 and, due to their high PZC, both kinds of particles were significantly positively charged. Therefore, an additional factor must have influenced polymerin sorption on different-sized Al_2O_3 particles. Polysaccharide, melanin and proteins are the main components of polymerin (Capasso et al., 2002a), which is rich in hydroxyl and carboxyl groups and phenol rings. Polymerin also contains paraffinic carbon moiety (i.e., long aliphatic CH_2 chains) as indicated by the absorption band at 2930 cm^{-1} (Kang and Xing, 2005) in the FT-IR spectra (Figure 3) and reported by Capasso et al. (2002a).

TABLE 2. Parameters of Freundlich and Langmuir Sorption Isotherms Fitting for Bi-Phase and Three-Phase Systems

		Freundlich Parameters			Langmuir Parameters		
Sorbents	**Sorbates**	**logK_f**	***N***	**R^2**	**S_m**	**K**	**R^2**
Al-Micro	Pol	2.043 ± 0.025	0.63** ± 0.02	0.969	2970 ± 80	0.02	0.990
Al-Nano	Pol	4.424 ± 0.029	0.43** ± 0.02	0.941	203800 ± 5300	0.08	0.994

					K_{oc} (L/Kg)		**K_d*[a] (L/Kg)**		**K_d1[b] (L/Kg)**		**K_d2[c] (L/Kg)**	
					C_e=0.1C_s	**C_e=0.01C_s**	**C_e=0.1C_s**	**C_e=0.01C_s**	**C_e=0.1C_s**	**C_e=0.01C_s**	**C_e=0.1C_s**	**C_e=0.01C_s**
Al-Micro	Phen	0.431 ± 0.039	1.06 ± 0.02	0.997			3.5					
Al-Nano	Phen	1.590 ± 0.024	1.04 ± 0.01	0.999			50.3					
Pol[d]	Phen	3.642 ± 0.090	0.88** ± 0.05	0.967	2640	3194						
Pol-Al-Micro T[e]	Phen	1.453 ± 0.018	0.98 ± 0.01	0.999			14.6		24.0		20.2 (Koc=2609)	
Pol-Al-Micro C[f]	Phen	1.370 ± 0.021	0.97 ± 0.01	0.998			11.4		18.8 (Koc=2106)		14.9	
Pol-Al-Micro D[g]	Phen	1.365 ± 0.018	0.97 ± 0.01	0.999			11.6		19.0 (Koc=2204)		15.2	
Pol-Al-Nano T[e]	Phen	3.019 ± 0.032	0.96 ± 0.02	0.995			299		528		478 (Koc=1951)	
Pol-Al-Nano C[f]	Phen	3.051 ± 0.021	0.93** ± 0.01	0.997			331	391	523 (Koc=2545)	617.0 (Koc=2184)	479	570
Pol-Al-Nano D[g]	Phen	3.270 ± 0.011	0.98 ± 0.01	0.999			660		1080 (Koc=4734)		1030	

**Nonlinear sorption; K_d*[a] is the apparent K_d in a three-phase system; K_d1[b] is the corrected K_d by subtraction of C_b from the solution; K_d2[c] is the corrected K_d by subtraction of the amount of Phen sorbed on free particles. Pol[d] is the abbreviation of polymerin; [e] T is the sample obtained by mixing Phen, Pol and Al_2O_3 simultaneously; [f] C is the sample obtained by forming the complex first and then adding Phen in the solution; [g] D is the sample obtained by freeze-drying the pre-formed complex and then adding Phen.

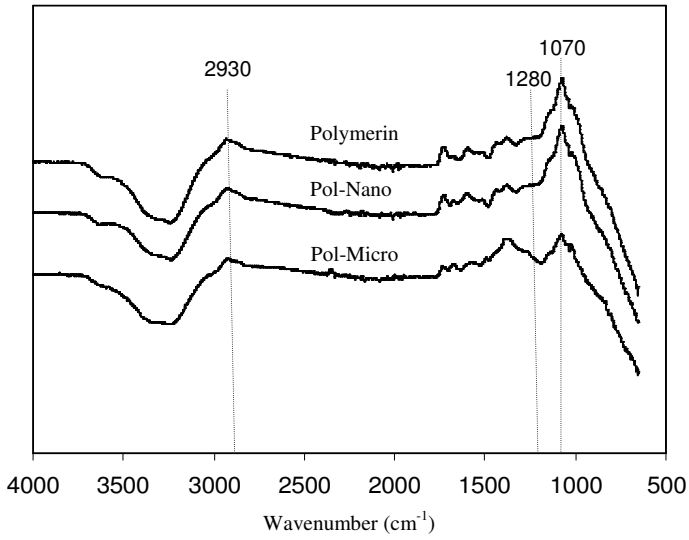

Figure 3. FT-IR spectra of original polymerin and polymerin solution remaining after adsorption on Al_2O_3 micro (Pol-Micro) and nanoparticles (Pol-Nano). Absorption band at 2930 cm^{-1} is assigned to paraffinic carbon moiety (i.e., long aliphatic CH_2 chains).

Reaching a microparticle, polymerin could find a wide surface to interact with. Each macromolecule may bind a microparticle using multiple functional groups from the same unit, spreading itself on the surface, assuming a *flat configuration* (Figure 4a). With regard to nanoparticles, each polymerin macromolecule may find a limited area on the highly charged surface. Polymerin macromolecules may have only one or few anchor points on the surface, but they must extend their branches radially from the central focus of the particle toward the solution because they do not have enough space on the surface. Since the charge density is high, several macromolecules are attracted by the mineral surface, forming a *radiant configuration* (Figure 4b). Moreover, after the first layer, other macromolecules can be adsorbed via both polar and

hydrophobic interactions. This *radiant configuration*, along the contribution from the higher surface area and zeta potential, can explain such a large variation of sorption capacity between different sizes of Al_2O_3.

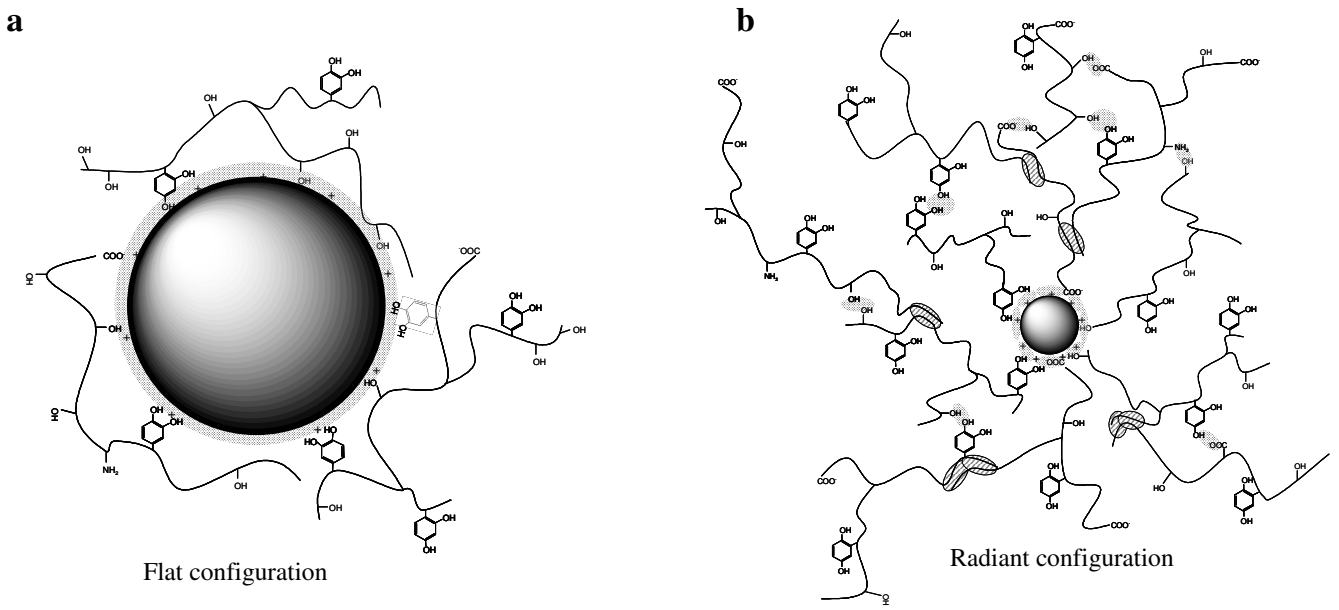

Figure 4. Schematic illustration of the hypothetic interaction between polymerin and aluminum oxides. The first panel (a) represents the hypothetic *flat configuration* of polymerin on micro-Al_2O_3; the second panel (b) represents the *radiant configuration* of polymerin on nano-Al_2O_3. The curve lines represent polysaccharide and paraffinic carbon chains, light grey circles show polar interactions (e.g., H-bonding), hatched areas indicate hydrophobic interactions (domains). This is an illustrative representation not drawn to scale

5.3.2 Fractionation of Polymerin Adsorbed by Al_2O_3 Microparticles

UV-Visible analysis data (Figure 5) show that, at low polymerin concentration, E_4/E_6 ratio of the polymerin solution remaining after adsorption on both types of particles, especially for microparticles, was higher than that of original polymerin. The E_4/E_6 ratio is a parameter related to the aromaticity of dissolved organic matter.

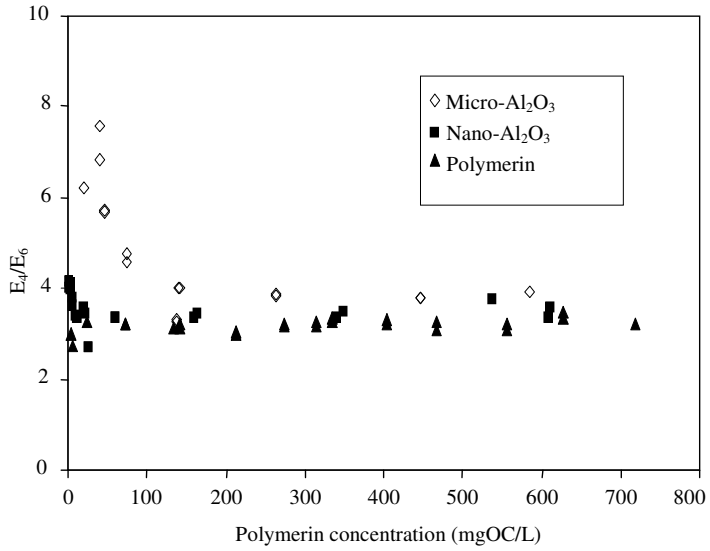

Figure 5. E_4/E_6 ratio of original polymerin and polymerin solution remaining after the interaction with micro and nanoparticles of aluminum oxides.

The higher value for polymerin solution remaining after adsorption by the particles is an evidence of fractionation occurring during sorption. Based on the notion that higher ratio may be related to lower aromaticity for the same organic matrix (Stevenson, 1994), this set of data showed that polymerin remaining after adsorption on aluminum oxides contained less aromatic components relative to

the original polymerin. Aromatic component is also related to lower molecular size, as reported by Reiller et al. (2006). By analyzing humic acid sorption on hematite using electrospray ionization with quadrupole time-of-flight mass spectrometry, Reiller et al. (2006) observed that the low molecular weight fraction was preferentially adsorbed, as well as the aromatic fraction. Moreover, Shin et al. (1999) and Kaiser (2003) demonstrated that the smaller sized fraction of humic acid is the one that has higher aromatic content and more acidic functional groups. Therefore, consistently with these previous studies, Al_2O_3 particles preferably adsorb the polar, aromatic components characterized by a low molecular size, leaving aliphatic fraction in solution. This evidence was stronger for microparticles in comparison with nanoparticles. Lower fractionation by nanoparticles is an additional evidence of its higher sorption capacity. Nanoparticles are able to adsorb polymerin components (including paraffinic carbons) indistinctly (Figure 3), without making any significant selection. With increasing polymerin concentration, E_4/E_6 ratio decreased until the same value of original polymerin. At this point, the high polymerin concentration in solution does not permit to discriminate the differences anymore.

The adsorption of the polar component on microparticles was also supported by FT-IR spectra, as shown in Figure 3. In the spectrum of polymerin solution remaining after adsorption on microparticles, the band at 1280 cm^{-1}, related with phenolic OH and C-O functional groups (Fu and Quan, 2006; Gu et al., 1995), disappeared and the band at 1070 cm^{-1}, assigned to polysaccharide alcoholic groups, drastically decreased.

5.3.3 Sorption of Phenanthrene on Each Single Sorbent

Sorption isotherms of Phen on aluminum oxides and polymerin are shown in Figure 6. Both micro and nanoparticles had an approximately linear sorption, with their *N* values being 1.06 and 1.04, respectively (Table 2). Nanoparticles had a sorption capacity much higher than microparticles, with a K_d value almost fifteen times greater than microparticle's K_d. Phen sorption on polymerin fit the Freundlich equation. Polymerin sorption capacity was more than two orders of magnitude higher than nanoparticles' capacity.

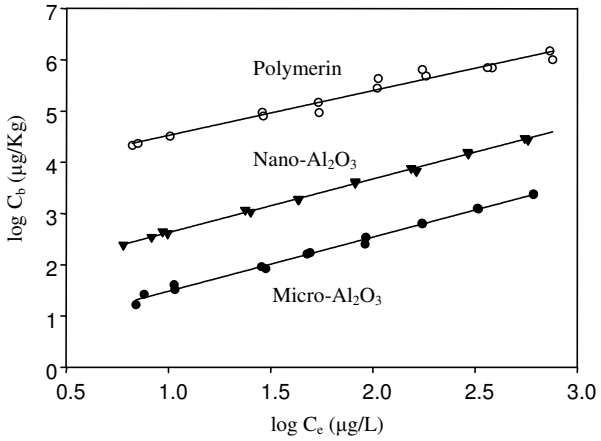

Figure 6. Sorption isotherms of Phen by polymerin, micro-Al_2O_3 and nano-Al_2O_3.

5.3.4 Effect of Polymerin Concentration on Apparent Sorption Coefficient (K_d) of Particles

Phen sorption on particles in the presence of increasing concentration of polymerin was studied by keeping a constant Phen concentration and the quantity of aluminum oxides. Apparent K_d of Phen was highly enhanced by increasing added polymerin (Figures 7a and 7b). The high initial enhancement was related with the increasing of organic carbon complexed on the particles. In reality, both aqueous and solid polymerin concentrations increased, therefore Phen sorption on solid particles was affected by the balance between these two phases. The increase of the sorption in the beginning of the curve showed the complex as the dominant sorbent. Many studies (Xiao et al., 2004; Gregory et al., 2005) reported that the higher SOM in a soil-water system, the higher PAHs sorption. Once polymerin reached the saturation point on the nanoparticles, the apparent K_d started to slightly decrease by increasing polymerin concentration (Figure 7b). That is easily explainable by comparing K_d and K_{OC} of Phen on the particles alone and polymerin, respectively. Polymerin-particles complex showed higher Phen sorption than the oxides alone, and the sorption intensified by increasing the organic percentage of the complex. Once the complex is saturated and no more polymerin can be adsorbed, the organic fraction in solution compete with the complex for Phen sorption. This behavior was only evident for nanoparticles. Microparticles showed a constantly increasing trend, probably because these particles did not reach a polymerin saturation point in the examined polymerin concentration range. In fact, by considering the last polymerin equilibrium concentration (Figures 7a and 7b) for both micro and nanoparticles (450 and 600 $mgOC \cdot L^{-1}$, respectively), the adsorbed amount, calculated by using Langmuir parameters, are 2670 and 199600 $mgOC \cdot Kg^{-1}$.

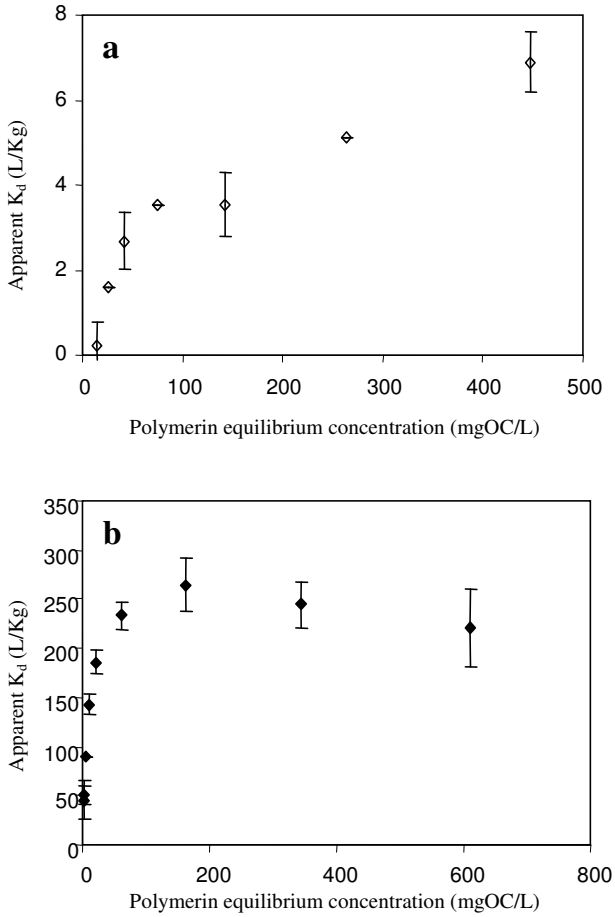

Figure 7. Variation of the apparent sorption coefficient (K_d) in a three-phase system using a fixed concentration of phenanthrene and increasing polymerin amount in solution with Al_2O_3 microparticles (a) and nanoparticles (b).

Polymerin adsorbed amount on microparticles is significantly different from its maximum sorption capacity (S_m = 2970 ± 80), thus, polymerin did not reach the saturation point, differently from what occurred for nanoparticles (S_m = 203800 ± 5300).

5.3.5 Sorption Sequence Influenced Microparticles Behavior

Phen sorption in a three-phase system was studied by keeping a constant polymerin concentration and varying Phen amount.

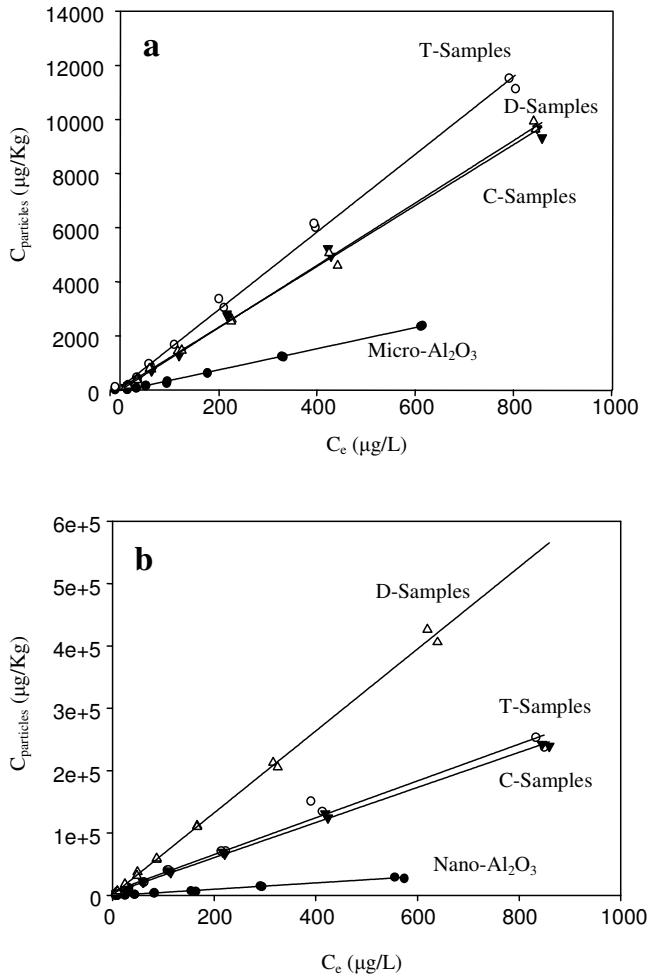

Figure 8. Sorption isotherms of phenanthrene in a three-phase system using Al_2O_3 microparticles (a) and nanoparticles (b) in different experimental conditions (see Figure 1).

Moreover, the effect of sorption sequence and the influence of the sorbent physical state were investigated by following the experimental design shown in Figure 1.

Microparticles showed a sorption coefficient following an order: T-samples (20.2) > C-samples (15.0) ≈ D-samples (15.2), with a T-samples K_d2 value 34% higher than the others (Table 2). All the isotherms presented a slightly nonlinear shape with N values ranging from 0.97 to 0.98. The effect of sorption sequence significantly influenced Phen behavior in a three-phase system. An higher sorption coefficient was registered when Phen and polymerin were added simultaneously into the system (Figure 8a). This was a clear evidence of the competition occurring between Phen and polymerin for the sorption sites on the particles. In T-samples all the particles sorption sites were available for both sorbates (Phen and polymerin), but in C- and D-samples oxides were coated by polymerin, hence the only available sorption sites for Phen were on polymerin-particles complex or on un-adsorbed polymerin in solution.

By summing the isotherm resulted from both microparticles alone and C- or D-samples, the obtained curve could overlap to the T-samples curve (Figure 9). This mathematical calculation demonstrated the contribution of both particles and complex in a three phase system. After complex formation, oxides contribution to sorption of Phen was negligible. Huang and Weber (1997) reported that minerals do not play a significant role in PAHs sorption in soil/sediment-water system. Kile et al. (1995) suggested the occurring of a strong suppression by water of solute adsorption on polar mineral surfaces.

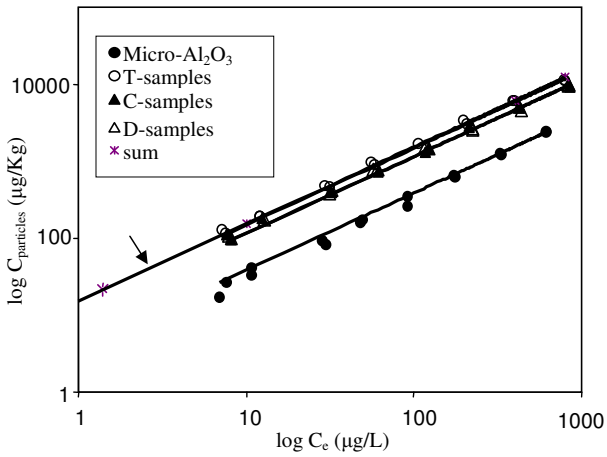

Figure 9. Sum of the isotherms of phenanthrene on aluminum microparticles alone and C- (or D-) samples. The line, pointed with an arrow, overlap the isotherm of T-samples.

Moreover, sorption of hydrophobic compounds from aqueous solutions by soil and sediments is dominated by sorption in soil organic matter. By analyzing our results, in addition to the above reasons we can contend that minerals contribution to sorption of PAHs in a complex system is also insignificant because their surface sites are preferentially covered by organic matter and/or water.

5.3.6 The Drying Treatment Increased the Sorption of Phenanthrene by Nanoparticles

Nanoparticles showed a sorption coefficient following an order different from microparticles: D-samples (1030) >> T-samples (478) ≈ C-samples (480-570), with a D-samples K_d2 value being two times higher than the others. T- and D-samples presented a linear sorption, while C-samples isotherm was nonlinear, with *N* value of 0.93 (Table 2).

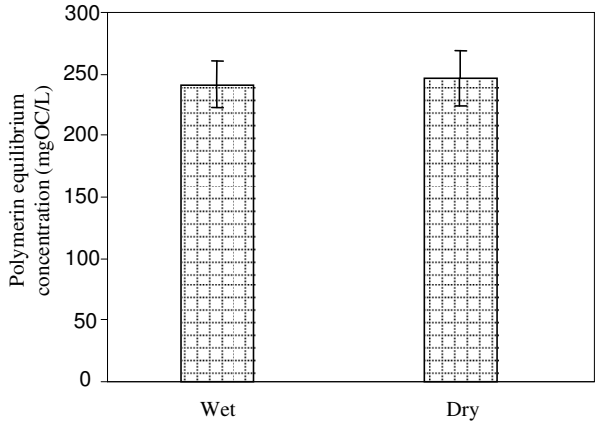

Figure 10. Histograms showing polymerin equilibrium concentration in a three-phase system in C-samples (Wet) and in D-Samples (Dry). Polymerin concentration was measured at the end of the experiment for both batches (6 days, see experimental design, Figure 1). This graph shows that there is no significant difference ($p<0.05$) between C- and D-samples in terms of polymerin adsorbed on Al_2O_3 nanoparticles.

The addition of Phen and polymerin simultaneously or in two successive times was not crucial for Phen sorption in a nanoparticles three-phase system (Figure 8b). T- and C-samples showed similar K_d values. This result means that polymerin affinity for nanoparticles was much stronger than Phen affinity.

Critical point for Phen sorption was the physical state of the complex. Dried samples showed a K_d value twice higher than the other systems. Since there was no difference between wet and dry complexes in terms of polymerin adsorption on the particles (Figure 10), this result can be explained according to the *radiant configuration*, as suggested in Figure 11. In C-samples, polymerin chains extended their branches in solution, exposing hydrophilic domains and leaving the lipophilic ones relatively inside. During the freeze-dry process, water molecules gradually left from the complex changing the chain configuration. Water molecules on the surface left faster than the ones inside the complex. Therefore, hydrophilic groups of polymerin would rearrange to contact with the water molecules inside. This rearrangement led polymerin branches to fold, exposing a relatively lipophilic surface and creating hydrophilic domains relatively inside the matrix (D-samples, Figure 11). Effect of drying on the increase in hydrophobicity of the surfaces of soil particles was recently described by Klitzke and Lang (2007). Moreover, this drying process created additional lipophilic sites relative to the C-samples, increasing the affinity for Phen. Furthermore, sorption linearity for D-samples was slightly higher than for C-samples, indicating that the increase of hydrophobic regions led to an increase of a more partitioning process. Another important result was that a dried nanoparticles complex showed a sorption coefficient nearly 70 times higher than a dried microparticles complex (Table 2), making the former more eligible than the latter for the application as bio-filter in water treatment.

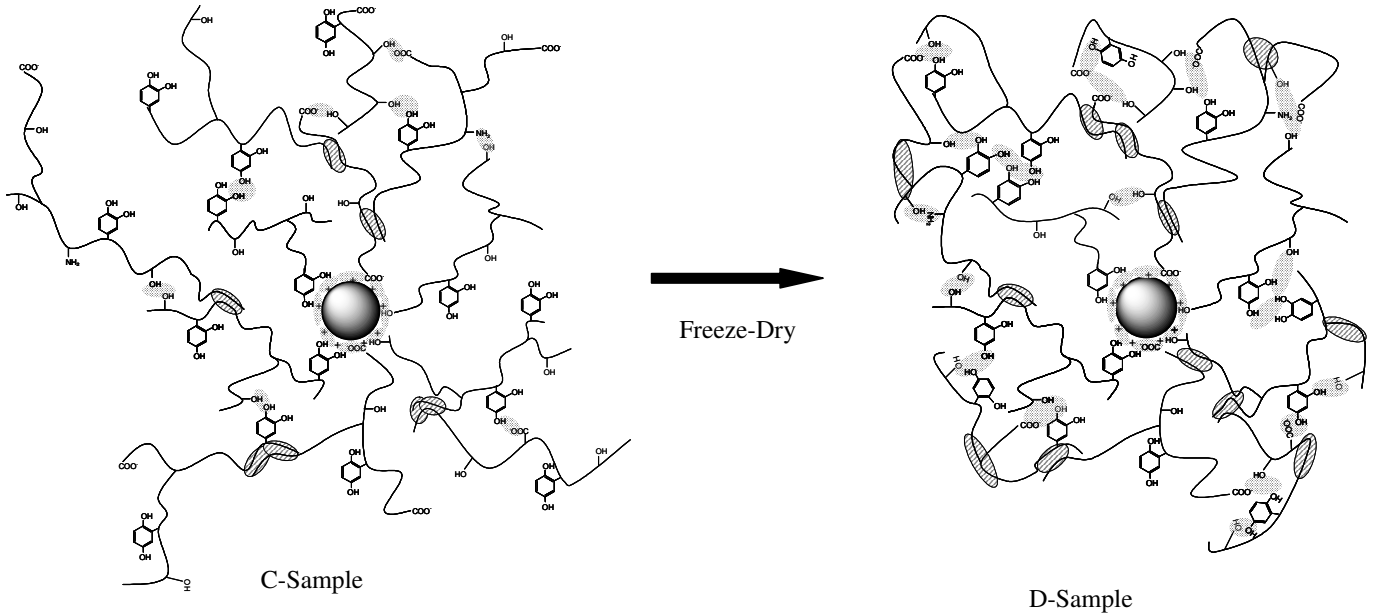

Figure 11. Schematic illustration of the hypothetic interaction between polymerin and aluminum oxides. The drawing shows the conformational change of polymerin chains occurring after the drying treatment on nanoparticles of aluminum oxides. The curve lines represent polysaccharide and paraffinic carbon chains, light grey circles show polar interactions (e.g., H-bonding), hatched areas indicate hydrophobic interactions (domains). This is an illustrative representation not drawn to scale.

5.4 Conclusions

Polymerin sorption on alumina significantly depends on particles size. The higher surface area and zeta potential of nanoparticles compared to microparticles partially increased polymerin sorption. However, a different configuration of polymerin molecules on the particle surface may be the effective cause of a large enhancement of the sorption on nanoparticles. UV-Visible and FT-IR analyses demonstrated polymerin fractionation by Al_2O_3 microparticles, which preferentially adsorbed the polar, aromatic fraction. In a three-phase system with a varying amount of polymerin, Phen sorption directly depended on the carbon content of the complex. Using a fixed polymerin concentration, Phen sorption on microparticles was affected by the sorption sequence. The highest sorption was registered by adding Phen and polymerin simultaneously on the Al_2O_3 particles. Nanoparticles behavior was not influenced by the sorption sequence, but by the physical state of the complex. The drying treatment considerably enhanced Phen sorption. These findings support the application of polymerin, especially associated with Al_2O_3 nanoparticles in a dry complex, for removing HOCs from point source polluted water. Polymerin represents a cheap sorbent and its use could minimize OMW disposal problem. Thus, use of polymerin in water treatment could give both economic and ecologic advantages.

CHAPTER 6

Overall Conclusions

The application of polymerin, the polymeric organic fraction recovered from olive mill wastewater, as a bio-filter for remediation of point source-polluted waters was investigated. Paraquat and 2,4-D were used as model of ionic herbicides. The capability of polymerin at native pH to totally remove paraquat from simulated wastewaters identifies this bio-sorbent as a bio-filter suitable in water treatment. Being an anionic polyelectrolyte, polymerin may also be versatile for the removal of other cationic pesticides. Decontamination from 2,4-D was significant but incomplete, indicating the existence of a threshold concentration value (0.04 $mmol \cdot L^{-1}$) for this herbicide at which no sorption occurred, and this value was independent on the amount of sorbent and the number of cycles. In this case, the immobilization of polymerin on a cationic sorbent (i.e., ferrihydrite or aluminum oxide) may be necessary for the total removal of the pesticide from polluted waters. As a consequence of the sorption, either for paraquat or 2,4-D, it is possible to find a release from the sorbent of K, Mg and Ca, which are macro-elements essential in plant and animal nutrition. Thus, this kind of water can be potentially recycled for irrigation in the same rural zones where point source pollution occurs. The application of the methodology to cyhalofop acid, that is a new generation pesticide, showed that

polymerin was able to totally remove it from simulated wastewaters, encouraging the use of this bio-material as a tool limiting a real environmental risk.

With this study we also demonstrated that polymerin is able to adsorb phenanthrene, which is a completely apolar pollutant. Moreover, the association of polymerin with Al_2O_3 nanoparticles, especially in a dry complex, showed excellent results, matching the high sorbing capacity of an organic matrix with the better manageability of a solid sorbent.

Summarizing, we proved the capability of polymerin to remove polar organic pollutants from contaminated waters. In addition, polymerin sorption of phenanthrene opens new horizons in the use of this sorbent for the decontamination of waters polluted by a wide range of chemicals. Thus, polymerin may be used alone as a component in the biobeds and loaded on Al_2O_3 nanoparticles as a bio-filter for wastewater treatment. Moreover, the use of polymerin minimizes the disposal problem of olive mill wastewaters, making this waste a source of useful bio-materials.

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